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## Introduction

The cryptophycins are antimitotic, cyanobacterial metabolites isolated from the blue-green algae *Nostoc* sp. GSV 224. Preliminary *in vivo* studies showed that the cryptophycins are active against breast, pancreatic and colon tumors,<sup>1</sup> and retain their potency against the multi-drug resistant cancer cell lines.<sup>2</sup> Cryptophycin-52, the C6 *gem*-dimethyl analogue of cryptophycin A, is currently in clinical trials.<sup>3</sup> Although cryptophycin A (**1**) was originally introduced as a microtubule destabilizing agent,<sup>1,4,5</sup> its mechanisms of action have grown to include inhibition of the GTPase that regulates hydrolysis of GTP at the microtubule ends,<sup>6</sup> activation of the caspase (ICE/CED-3 protease) cascade,<sup>7</sup> slowing of the microtubule dynamics,<sup>8</sup> induction of apoptosis,<sup>7</sup> and induction of bcl-2 hyperphosphorylation.<sup>9</sup> To date, structure-activity relationship studies regarding the C3 and C10 side chains have not been fully probed. Although analogues were isolated containing varied side chains at C3, no isolation or synthesis of the C3 epimer has been reported. The synthesis of analogues containing an inversion of the C3 center has been carried out to determine if the natural C3 stereochemistry is required for pM activity and to gain information about the orientation of the hydrophobic C3 binding site on tubulin. Regarding the C10 side chain, the necessity of the aromatic oxygen has not been fully studied. The synthesis of analogues which contain varied substitution patterns on the C10 side chain, selected using the Topliss-Martin operational scheme,<sup>10</sup> are being prepared. *In vitro* biological testing has been performed to determine the biological activity in selected breast cancer cell lines and in a tubulin assay. The studies outlined in this research summary will provide insight into how the changes in the structure of cryptophycin A effect its ability to interact with tubulin and elicit cytotoxic effects.

## Body

### I. Introduction

Cryptophycins are antimitotic,<sup>1,11,12</sup> cyanobacterial metabolites isolated from the blue-green algae *Nostoc* sp. GSV 224.<sup>2,12,13</sup> A close structural relative, arenastatin A (2), differs from cryptophycin A (1) in that it lacks both the chlorine on the substituent at C10 and the methyl group at C6 (Figure 1). *In vivo* studies showed that the cryptophycins are active against breast, pancreatic and colon tumors, and retain their potency against multi-drug resistant cancer cell lines.<sup>2</sup> Cryptophycin-52 (3), the C6 *gem*-dimethyl analogue of cryptophycin A (1), is currently in clinical trials.<sup>3</sup> Although cryptophycin A (1) was originally introduced as a microtubule destabilizing agent,<sup>1,4,5</sup> its mechanisms of action have grown to include inhibition of the GTPase that regulates hydrolysis of GTP at the microtubule ends,<sup>6</sup> activation of the caspase (ICE/CED-3 protease) cascade,<sup>7</sup> slowing of the microtubule dynamics,<sup>8</sup> induction of apoptosis,<sup>7</sup> and induction of bcl-2 hyperphosphorylation.<sup>9</sup> To date, the SAR regarding the stereochemistry of the C3 side chain and the necessity of the aromatic oxygen on the C10 side chain have not been fully probed. Therefore, analogues containing various substitution patterns on the C10 side chain, and analogues with the inverted C3 center have been prepared.

Currently in the Georg group, we hypothesize that the C3 center must be in the natural *S* configuration in order for favorable interaction between a hydrophobic pocket of the binding site and the C3 isobutyl group to occur. If this is valid, then the C3 analogues will not be as cytotoxic as cryptophycin A (1). Of all of the analogues isolated by Moore and coworkers,<sup>14</sup> none contained the inverted C3 stereocenter, and to date no synthesis of such compounds have been published. I have proposed that by inverting the stereochemistry at C3, analogues 4 and 5 in Figure 2, information regarding the orientation of the hydrophobic binding site will be gained along information about the stereochemical requirement of the C3 center for activity.

SAR studies by Moore *et al.*<sup>14</sup> revealed that on the C10 side chain, removal of the C10 aryl C3' chlorine or the C4'-*O*-methyl group caused the loss of *in vivo* activity<sup>7</sup> (see Figure 1 for numbering system). After preliminary biological testing of 7, which contains the C3'-chloride, revealed that it was only 5-fold less active than cryptophycin A (1), we proposed to synthesize a family of analogues (6-9) to better define the SAR surrounding the C10 phenyl ring. These analogues were chosen using the Topliss-Martin operational scheme<sup>10</sup> which was developed to optimize the activity of a compound by altering the substitution pattern on a benzene ring (Scheme 9).

The analogues of interest, 4 - 9, all lack the C6 methyl group which is not necessary for *in vitro* activity, but is required for *in vivo* activity where it slows hydrolysis of the ester bond. Since all analogues will be screened for biological activity in an *in vitro* tubulin assay, and *in vitro* cell line assay the analogues lacking the substitution at C6 have been selected for synthesis and testing.

Initially, the biological testing has been completed using the diastereomeric mixture (~2:1 ratio  $\beta$ : $\alpha$ ) of the epoxides for analogues 4-9. Because separation of the mixture can only be achieved using high pressure liquid chromatography (HPLC),<sup>1</sup> purification will be completed in the future. Because it was previously determined that the  $\beta$  epoxide is the active cryptophycin analogue,<sup>15</sup> the  $\alpha$  and  $\beta$  analogues of 4-9 will be retested individually in the *in vitro* tubulin assay and the breast cancer cell line assay. The biological results obtained from testing the mixtures of epoxides has provided preliminary information regarding the range of concentration in which the analogues are active.

Arenastatin A (2) and the analogues proposed were all prepared using a similar synthetic approach. The retrosynthetic analysis of arenastatin A (2), depicted in Scheme 1, outlines the division of the molecule into two key fragments: the "northern half" 10, and the "southern half" 11. The northern half is obtained through asymmetric synthesis, while the southern half is formed through the coupling of three easily accessed building blocks: 1) L-leucic acid (12), 2)  $\beta$ -alanine (13), and 3) a D-tyrosine derivative (14). The L-leucic acid and the  $\beta$ -alanine are commercially available, and the tyrosine moiety is easily derived in a few steps from D-tyrosine.

## II. Statement of Work Discussion

In the statement of work, five goals were proposed: (1) synthesis of the arenastatin analogues **4** and **5** with the inverted C3 center, (2) attempt to improve the synthetic route to the northern half **10**, (3) attempt to improve the final epoxidation step of the arenastatin A (**2**) and cryptophycin A (**1**) synthesis, (4) modification of the aryl ring at C10 (analogues **6-9**), and (5) modification of the aryl moiety in the side chain of C16.

### Goal 1: Synthesis of Analogues **4** and **5**

#### A) C3 Analogue Synthesis

Synthesis of **4** began in first year of this grant.<sup>16</sup> D-Leucine (**15**) was converted to D-leucic acid (**16**),<sup>17</sup> and taken on to afford acid **19a** (Scheme 2). Synthesis of **4** and **5** was completed during the second year of funding<sup>18</sup> (see April 2000 report for a depiction of the full synthetic route), but resynthesis of both analogues was necessary in order to obtain material for biological testing and for full characterization of the compounds. In the final year, removal of the palladium catalyst used to form **19a** and **19b** from **17a** and **17b**, respectively, proved to be problematic. Earlier the impurity had been removed in the subsequent step of the synthesis, but for full characterization, pure samples of acids **19a** and **19b** were required. Therefore, benzyl esters **18a** and **18b** were prepared and cleanly converted to acids **19a** and **19b** (Scheme 3). Although **5** was resynthesized and tested as a mixture of the  $\alpha$  and  $\beta$ -epoxides in the tubulin assay and in the *in vitro* cell line assay, preparation of **4** has not yet been completed due to the need to resynthesize **19a** via the benzyl ester route.

#### B) C3 Analogue Biological Results

Preliminary biological results of the  $\alpha$  and  $\beta$ -epoxide mixture of **5**, completed within the last year, are shown in Table 1. In the tubulin assay, preliminary results demonstrated that **5** was 5-fold less active than cryptophycin A (**1**). In an *in vitro* MCF-7 breast cancer cell line **5** was found to be active at 80 nM, 6,000-fold less active than cryptophycin A (**1**), and in the corresponding MCF-7/ADR MDR breast cancer cell line **5** was approximately 20,000-fold less active than cryptophycin A (**1**). Biological testing of 4-methoxy analogue **4** has not yet been completed. This preliminary data demonstrates that inversion of the C3 center provides an analogue that is less active than cryptophycin A (**1**) in the tubulin and the *in vitro* cell line assays studied. In the future, (HPLC)<sup>1</sup> purification will be completed and the  $\alpha$  and  $\beta$  analogues of **4** and **5** will be retested individually in the *in vitro* tubulin assay and the breast cancer cell line assay.

### Goal 2: Attempt to Improve the Synthetic Route to the Northern Half **10**

The second goal, improving the synthetic route to the northern half **10** of cryptophycin was pursued in the second year.<sup>18</sup> The original asymmetric synthesis of **10**, developed in the Georg laboratory, was completed using two key reactions, a Noyori asymmetric hydrogenation<sup>19</sup> which set the *S* configuration of the hydroxyl group (conversion of **22** to **23**, 63%, 97% ee, Scheme 4) followed by a Frater reaction<sup>20,21</sup> (conversion of **23** to **24**, 71%, 95% de, Scheme 4), which allowed for stereoselective introduction of the methyl group of **24**.<sup>22</sup> The overall yield of **10** for the eleven step synthesis, starting from the diketo ester **22**, was 5%.

As in many total syntheses, the need for more starting material is always present. Therefore, a second route more amenable to scale-up, was developed which uses the  $\beta$ -allyldiisopinocampheylborane (**26**) developed by H. C. Brown, to set both stereocenters of **10** in a single step (Scheme 5).<sup>18,23</sup> In the key step of the crotylboration route, aldehyde **25** was reacted with  $\beta$ -allyldiisopinocampheylborane **26**, derived from *trans*-2-butene and (-)- $\beta$ -methoxydiisopinocampheylborane, to provide olefin **27** in 76%, 91% de.<sup>18</sup> The overall yield of **10** for the nine step crotylboration route (not shown) starting from 1,3-propanediol was 17%.

### A) Attempt to Improve the Northern Half Synthesis<sup>18</sup>

During the development of the crotylboration route (Scheme 5), an alternative approach (Scheme 6) was pursued in which construction of the two asymmetric centers would be completed after the  $\alpha,\beta$ -unsaturated ester was established. This route was pursued during the second year of funding, in the hopes of simplifying the purification of **27** which was hampered by the borane byproduct which traveled closely to **27** during chromatography. By substituting another substrate for **25** in the crotylboration reaction depicted in Scheme 5, we hoped to obtain a product that was easier to purify. Three different synthetic routes to the new proposed substrate, **28**, were pursued (see April 2000 report for full details). In the first approach the synthesis of **28**, obtained from 1,3-propanediol (**31**), proved to be problematic due to trouble in the conversion of **32** to **28** (Scheme 7). A second route was devised in which intermediate **34** would be cleaved oxidatively to form **28**, but formation of **34** proved to be problematic. Therefore, a third route to formation of aldehyde **28** via a photochemical reaction of **35** was attempted, but aldehyde **28** proved to be highly unstable, and hard to isolate. Therefore, pursuit of an improved route to the northern half of arenastatin A (**2**) was halted at the end of the second year.

### Goal 3: Epoxidation Studies<sup>16</sup>

Epoxidation is the final step in the synthesis of the epoxide-containing arenastatin and cryptophycin compounds. Although numerous syntheses of both the arenastatins and cryptophycins have been published<sup>1,15,24-29</sup> the epoxide has been installed using *m*-CPBA or dimethyldioxirane. This is of significance because the two diastereomeric epoxides are formed, at best in a 3:1 ratio. Because these epoxides cannot be separated using flash column chromatography, it is necessary to use HPLC to separate them. HPLC is a time consuming procedure and is limited by the amount of compound that can be loaded onto a given column. Consequently, we pursued the stereoselective epoxidation of **36** (Scheme 8) during my first year under sponsorship.<sup>16</sup>

### A) Epoxidation Studies<sup>16</sup>

Scheme 8 depicts the alternative conditions tried to selectively form arenastatin A (**2**). Specifically, the use of the chiral dioxirane **37**, hindered dioxiranes **38** and **39**, and bulky peroxypivalic acid **40** were reviewed. None of the conditions afforded the desired selective epoxidation (see April 1999 report for full discussion). Therefore, after numerous trials, pursuit of this goal was halted after the first year.

Interestingly, at the 220th American Chemical Society National Meeting (August 20-24, 2000, Washington, D. C.), researchers from Eli Lilly<sup>30</sup> presented work regarding their use of **37** to selectively form **2**. Due to similar problems in the selective epoxidation of **36**, they performed epoxidation with **37** on an intermediate used in their synthesis of **10**.

### Goal 4: Modification of the Aryl Ring at C10

Originally, we proposed to make the 3'-Cl analogue **7** and the 3',5'-dichloro analogue (not shown). After reviewing the outstanding preliminary biological results of **7** in the beginning of the third year, we decided to pursue the synthesis of a family of C10 analogues **6-9** containing various substitution patterns on the phenyl ring. The alterations in the substitution pattern were based on Topliss-Martin operational scheme (Scheme 9)<sup>10</sup> which was developed to optimize the activity of a compound bearing a benzene ring by altering the substitution pattern on the ring. Based on the biological activity of the compounds synthesized, different substitution patterns are suggested which correlate the substituent's characteristics such as lipophilicity, sterics and polarizability with its activity. Following this scheme, the non-substituted phenylalanine derivative **6** and the 4-chlorophenylalanine derivative **8** could be prepared and tested for biological activity. The activity of the 4-chloro compound, either more, less or equiactive than **6**, would define the next derivative to be prepared and tested. We opted to prepare a few compounds (**2**, and **6-9**), suggested in the first four tiers of the Topliss-Martin scheme, in parallel to see if we could optimize the activity of arenastatin A (**2**). Specifically, we noted that four of the five analogues [**6**, **8**, **9** Table 2 and Figure 2, and **47**

(not shown)] in the first three tiers could be easily prepared using commercially available *N*-Boc protected acids (**41**, **42**, and **44** and **45** Table 2). The fifth analogue which contained *N*-Boc protected acid **43** which is not commercially available, corresponded to arenastatin A (**2**). The synthesis of arenastatin A (**2**) was already in progress, and the synthesis of the necessary D-tyrosine moiety is detailed in the 1999 annual report.<sup>16</sup>

### A) Synthesis

As noted in the synthesis of the C3 analogues (Scheme 2), removal of the palladium catalyst used to form acid **51**<sup>7</sup> (Scheme 10) was problematic. In the last year, benzyl esters **50** were prepared, transformed to pure **51** via hydrogenation, and taken on to afford **2**, **6-9** (Scheme 10).

The synthesis of the C10 analogues started with the protection of L-leucic acid (**12**) as its benzyl ether **48**, followed by coupling with *N*-Boc-protected  $\beta$ -alanine. Intermediate **49** was deprotected and subsequently joined to the desired tyrosine moiety (**41-43**, **45** or **46**), to afford **50**. After removal of the benzyl group of **50**, acid **51** was activated using the Yamaguchi reagent, 2,4,6-trichlorobenzoylchloride, and coupled to **10**.<sup>16</sup> The *N*-Boc group and the *tert*-butyl ester of **52** were simultaneously cleaved and the macrocycle was closed using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagent.<sup>9</sup> Epoxidation with *m*-chloroperbenzoic acid (*m*-CPBA) yielded mixtures of the  $\alpha$  and  $\beta$  epoxides of **2**, and **6-9**.

### B) Biological Results C10 Analogues

The preliminary biological results of the  $\alpha$  and  $\beta$ -epoxide mixture of **6-9**, completed within the last year, are shown in Table 1. All assays have been conducted using cryptophycin A (**1**) as a control. Preliminary biological testing revealed that **7** was two-fold more active than cryptophycin A (**1**) in the tubulin assay while analogues **6**, **8** and **9** were not active below 20  $\mu$ M. In the MCF-7 breast cancer cell line assay **7** was approximately 1.5-fold less active than cryptophycin A (**1**) and approximately 2-fold less active in the MCF-7/ADR MDR cell line. In the MCF-7 and MCF-7/ADR breast cancer cell lines **9** was active in the 100-600 pM range where as **6** and **8** were found to be active in the nM range. In the future, (HPLC)<sup>1</sup> purification will be completed and the  $\alpha$  and  $\beta$  analogues of **6-9** will be retested individually in the *in vitro* tubulin assay and the breast cancer cell line assay.

### Goal 5: Modifications of the C16 Side Chain Aryl Moiety

Due to the time required to complete goals 1 – 4, modification of the C16 aryl moiety of the side chain of C16 was not completed by the time this report was written. The studies will be completed with support from the NIH.

## Key Research Accomplishments

- Total synthesis of arenastatin A analogues **4** and **5** containing the inverted C3 center was completed and preliminary biological test of the mixture of  $\alpha$  and  $\beta$ -epoxides of **5** was conducted.
- An improved synthetic route to the northern half **10** was pursued, but no improvement over the crotylboration route (Scheme 5) was found.
- A study to improve the selectivity of the final epoxidation step was conducted (Scheme 8); no improvement of the 3:1 ratio of the  $\beta$ : $\alpha$  epoxides formation of arenastatin A (**2**) from desepoxy-arenastatin A (**36**) was observed.
- Total synthesis of arenastatin A (**2**) and C10 analogues **6-9** was completed. Preliminary biological testing (Table 1) of analogues **6-9** was conducted.

## Reportable Outcomes

POSTDOCTORAL FELLOWSHIP: Chemistry, Anticipated start date February 2002, The Scripps Research Institute, La Jolla, CA, Advisor: Dr. Dale L. Boger.

ORAL PRESENTATION: Buck, Suzanne B.; Georg, Gunda I. "Total Synthesis and Biological Evaluation of C3 and C10 Analogues of Cryptophycin A." 2001 Winter Conference, Medicinal and Bioorganic Chemistry Foundation, Steamboat Springs, CO, January 28 - February 2, 2001.

PUBLICATION: Eggen, M.; Mossman, C. J.; Buck, S. B.; Nair, S. K.; Bhat, L.; Ali, S. M.; Reiff, E. A.; Boge, T. C.; Georg, G. I. Total Synthesis of Cryptophycin-24 (Arenastatin A) Amenable to Structural Modifications in the C16 Side Chain. *J. Org. Chem.* **2000**, *65*, 7792-7799.

POSTER PRESENTATION: Buck, Suzanne B.; Georg, Gunda I. "Studies Towards the Total Synthesis of Cryptophycin A Analogues." 27<sup>th</sup> National Medicinal Chemistry Symposium, Kansas City, MO, June 13 - 17, 2000.

POSTER PRESENTATION: Buck, Suzanne B.; Georg, Gunda I. "Synthesis of C3 Epimer Analogues of Cryptophycin A." Era of Hope, Department of Defense Breast Cancer Research Meeting, Atlanta, Georgia, June 8 - 12, 2000.

DEGREE: M.S., Medicinal Chemistry, August 1999, Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas.

ORAL PRESENTATION: "Studies Towards the Total Synthesis of Cryptophycin A and Arenastatin A Analogues," July 9, 1999, National Institutes of Health Symposium given by NIH predoctoral trainees, Lawrence, Kansas.

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National Institutes of Health, National Cancer Institute  
Pharmacophore/Interactions with Tubulin of Cryptophycin  
The major goal of this project is to prepare cryptophycin affinity labels to characterize the cryptophycin binding site.

POSTER PRESENTATION: Buck, Suzanne B.; Georg, Gunda I., "Studies Towards the Total Synthesis of Cryptophycin A and Analogues." April 16-18, 1999, Presented at the 37<sup>th</sup> MIKI Medicinal Chemistry Meeting, Lawrence, Kansas.

LECTURE: "Studies Towards the Synthesis of Cryptophycin A and Arenastatin A Analogues." October, 29, 1998, Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas.

POSTER PRESENTATION: Hanna, Suzanne B.; Eggen, MariJean; Mossman, Craig J.; Reiff, Emily A.; Boge, Thomas C.; Georg, Gunda I., "Studies Towards the Total Synthesis of Cryptophycin A and Analogues." May 22-24, 1998, Presented at the 36<sup>th</sup> MIKI Medicinal Chemistry Meeting, Chicago, Illinois.

## Conclusions

The preliminary results obtained from the mixture of  $\alpha$  and  $\beta$ -epoxides of **5** revealed that inversion of the C3 center does not produce an analogue that is equipotent to cryptophycin A (**1**) in the *in vitro* breast cancer cell lines assay or tubulin assay. Therefore, this supports the theory that the natural *S* configuration is required for pM activity, and inversion of this center must have disrupted the interaction between the hydrophobic pocket of the binding site and the C3 isobutyl group.

The alterations in the substitution pattern of **6-9** were based on Topliss-Martin operational scheme (Scheme 9)<sup>10</sup> which was developed to optimize the activity of a compound bearing a benzene ring by altering the substitution pattern on the ring. Based on the biological activity of the compounds synthesized, different substitution patterns were suggested which should correlate the substituent's characteristics such as lipophilicity, sterics and polarizability with its activity. We expected the biological results obtained would correlate well with the Topliss-Martin operational scheme, but unfortunately, we found just the opposite; the non-substituted phenylalanine analogue **6**, and the 4'-chloro analogue **8** were inactive below 20  $\mu$ M in the cell line assay, and the 3-chloro and 3',4'-dichloro analogues (**7** and **9**, respectively) were active in the pM range in the breast cancer cell line assay. Reviewing the Topliss-Martin scheme, one realizes that analogues **7** and **9** are located on opposite ends of the operational scheme. Had we followed the scheme directly, and prepared and tested analogues **6** and **8** first and reviewed their activity as compared to **7** (which had originally been synthesized, found to be potent, and produced interest in using the Topliss-Martin scheme), we most likely would have concluded that we had obtained the optimized analogue **7**, since both **7** and **2** are located together on the left side of the Topliss-Martin scheme. Interestingly, 3',4'-dichloro analogue **9** was also found to be potent in the pM range, which suggests that there is a very specific interaction between the C3' site in the 10 side chain and cryptophycin's binding site.

The implications of this work could extend to the development of other analogues for clinical trials in the treatment of breast cancer. To date, only cryptophycin-52 (**3**) is in clinical trials, but should this drug fail to make it to the market, other analogues would be pursued which have improved characteristics. This study has helped to further define the structure-activity relationship surrounding the C3 and C10 side chains. Future studies will be directed towards development of radiolabelled and photoaffinity labeled analogues that could be used in biological studies to provide more information regarding the binding sites of cryptophycin on tubulin and bcl-2 which are known cryptophycin targets.

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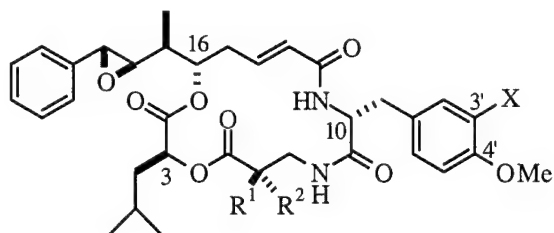
## Appendix

### Part A - Acronyms

BINAP - binaphthyl  
Bn - benzyl  
BnBr - benzyl bromide  
Boc - *tert*-butoxycarbonyl  
*m*-CPBA - *m*-chloroperbenzoic acid  
CH<sub>2</sub>Cl<sub>2</sub> - dichloromethane  
CH<sub>3</sub>OH - methanol  
CH<sub>3</sub>CN - acetonitrile  
DCC - 1,3-dicyclohexylcarbodiimide  
de - diastereomeric excess  
DIEA - diisopropyl ethylamine  
DMAP - 4-dimethylamino pyridine  
EDCI - 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride  
ee - enantiomeric excess  
EtOAc - ethyl acetate  
Et<sub>2</sub>O - diethyl ether  
GTP - guanosine triphosphate  
HBTU - *O*-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate  
HMPA - hexamethylphosphoramide  
HPLC - high pressure liquid chromatography  
LDA - lithium diisopropyl amide  
MeI - methyl iodide  
MDR - multidrug resistant  
[O] - oxidation  
RT - room temperature  
SAR - structure-activity relationship  
TBD - to be determined  
TFA - trifluoroacetic acid  
THF - tetrahydrofuran  
UV - ultraviolet  
Yamaguchi reagent - 2,4,6-trichlorobenzoyl chloride

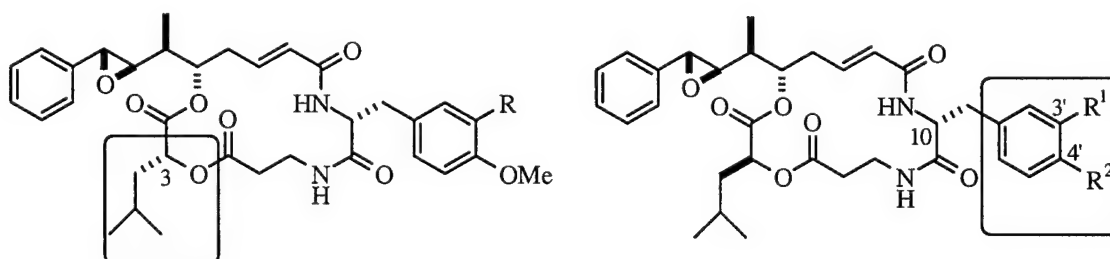
## Appendix Part B - Figures

**Figure 1.** Structures of cryptophycin A, arenastatin A and cryptophycin-52.



cryptophycin A (1), X = Cl; R<sup>1</sup> = Me; R<sup>2</sup> = H  
 arenastatin A (2), X = R<sup>1</sup> = R<sup>2</sup> = H  
 cryptophycin-52 (3), X = Cl; R<sup>1</sup> = R<sup>2</sup> = Me

**Figure 2.** Structure of C3 and C10 Analogues.

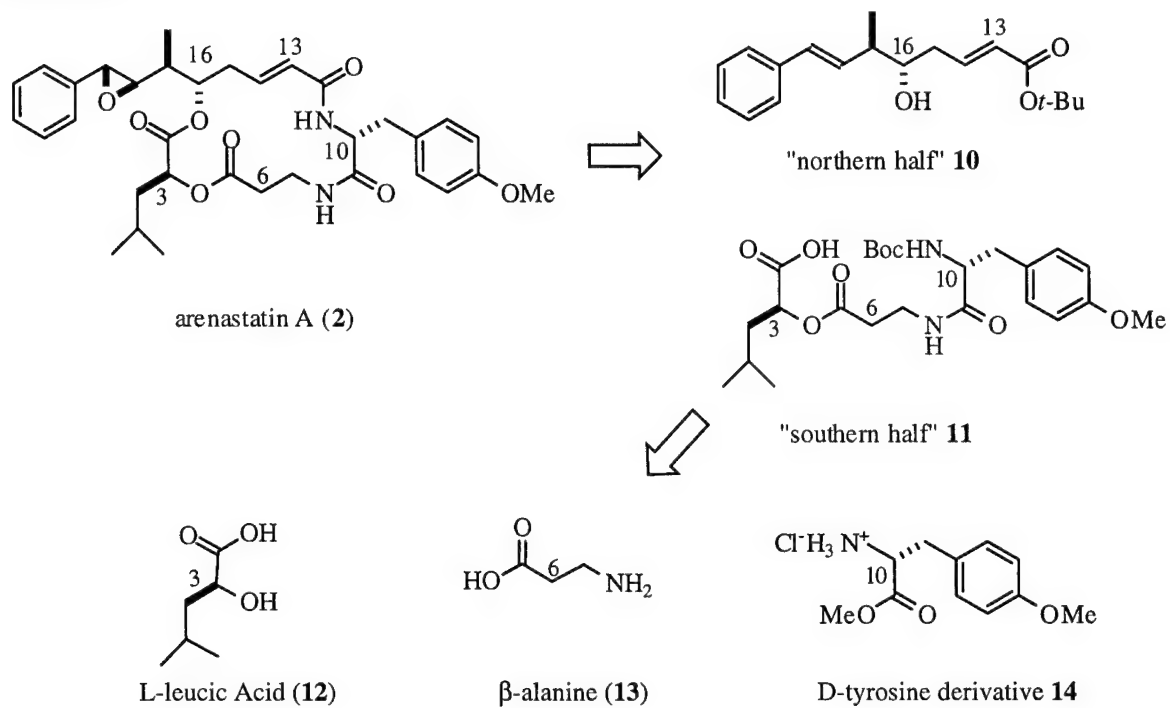


4, R = H  
 5, R = Cl

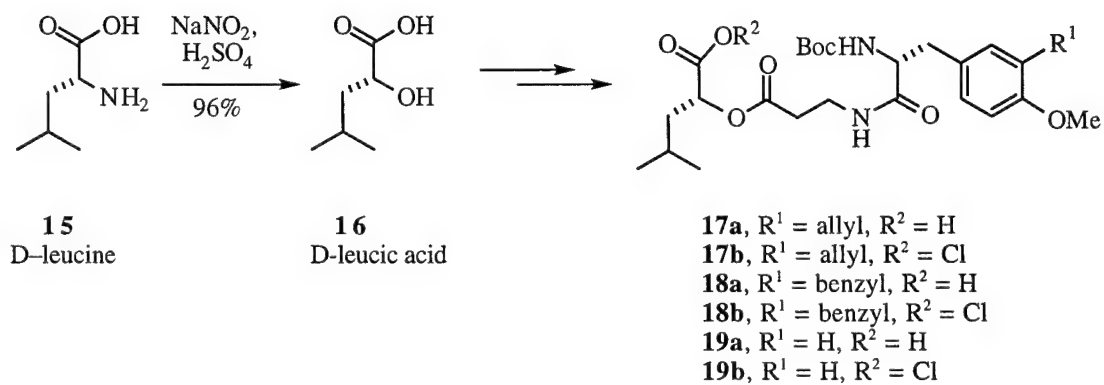
6, R<sup>1</sup> = R<sup>2</sup> = H  
 7, R<sup>1</sup> = Cl, R<sup>2</sup> = H  
 8, R<sup>1</sup> = H, R<sup>2</sup> = Cl  
 9, R<sup>1</sup> = R<sup>2</sup> = Cl

## Appendix Part C – Schemes

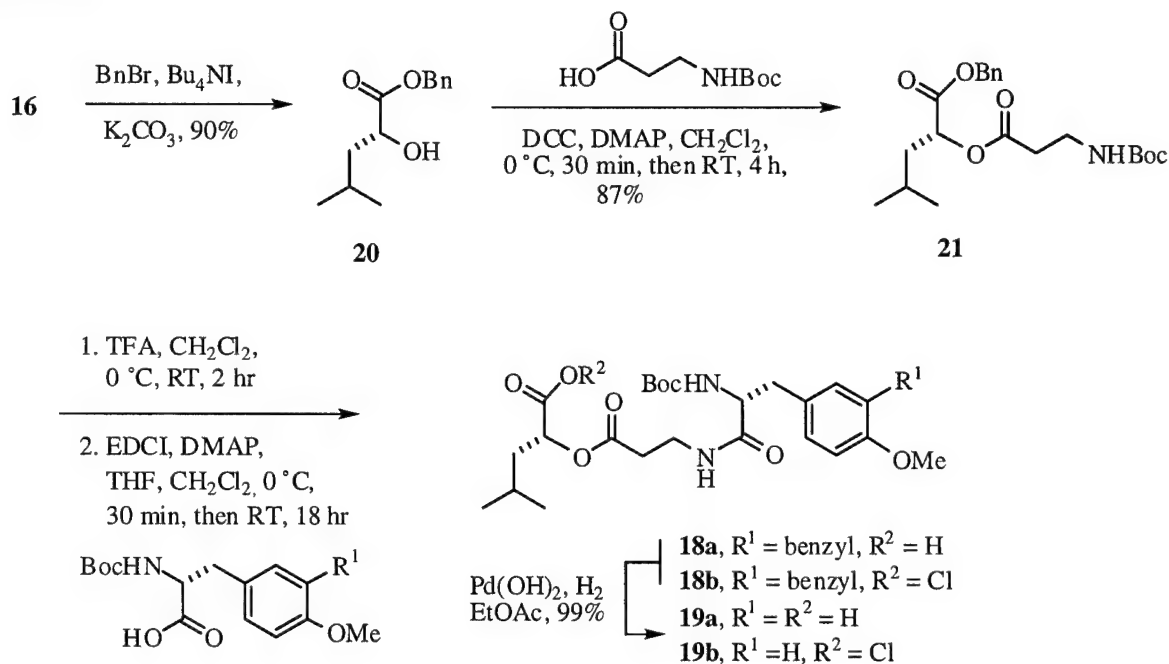
Scheme 1



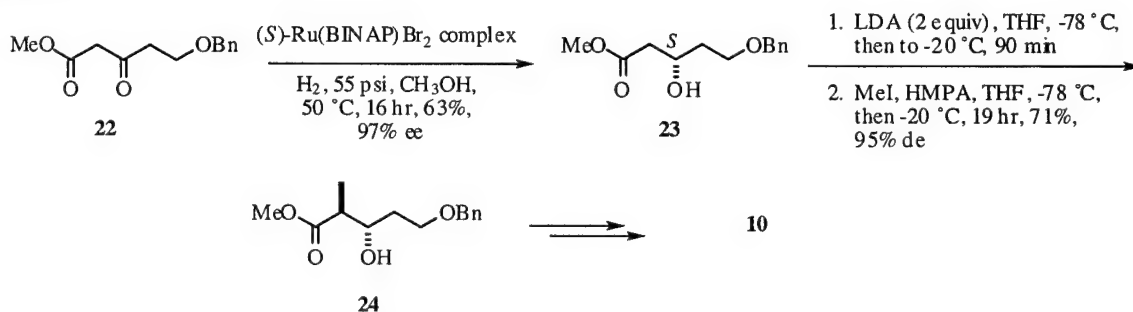
Scheme 2



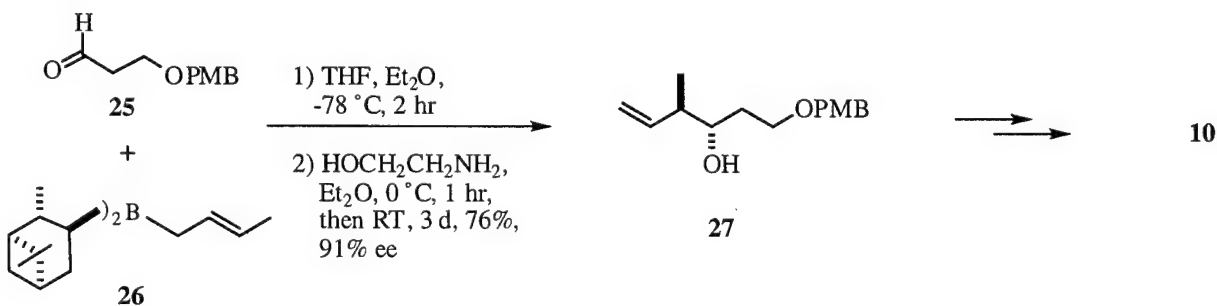
### Scheme 3



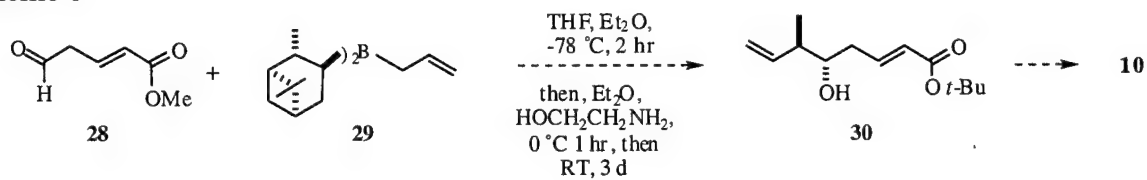
### Scheme 4



### Scheme 5

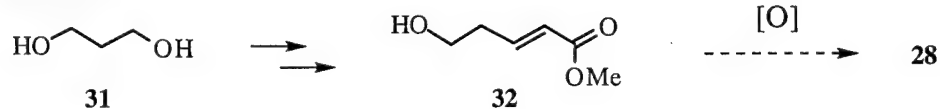


### Scheme 6

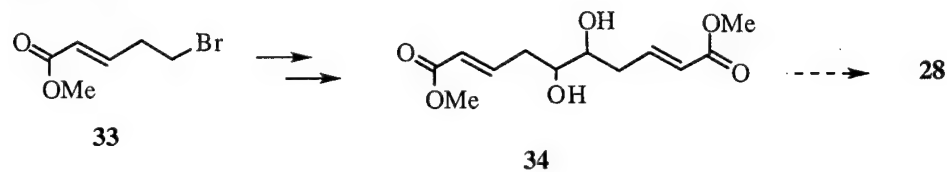


### Scheme 7

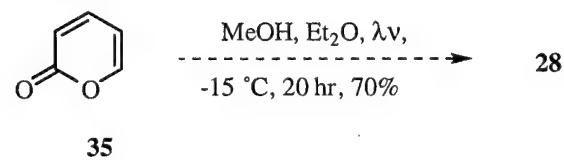
#### Route 1



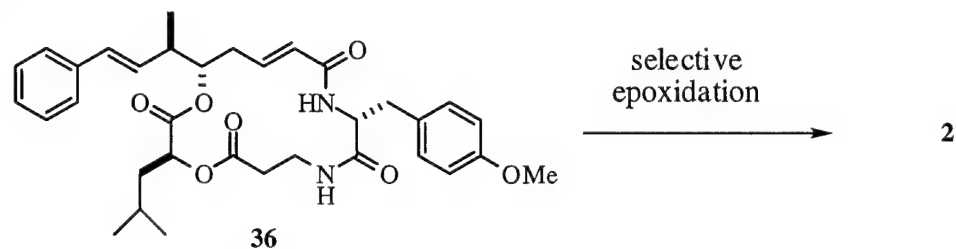
#### Route 2



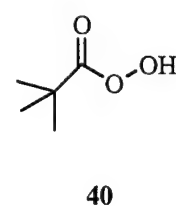
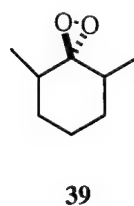
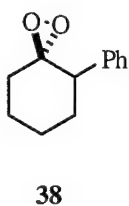
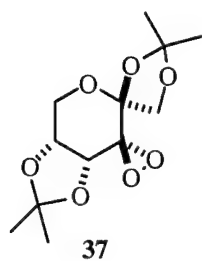
#### Route 3



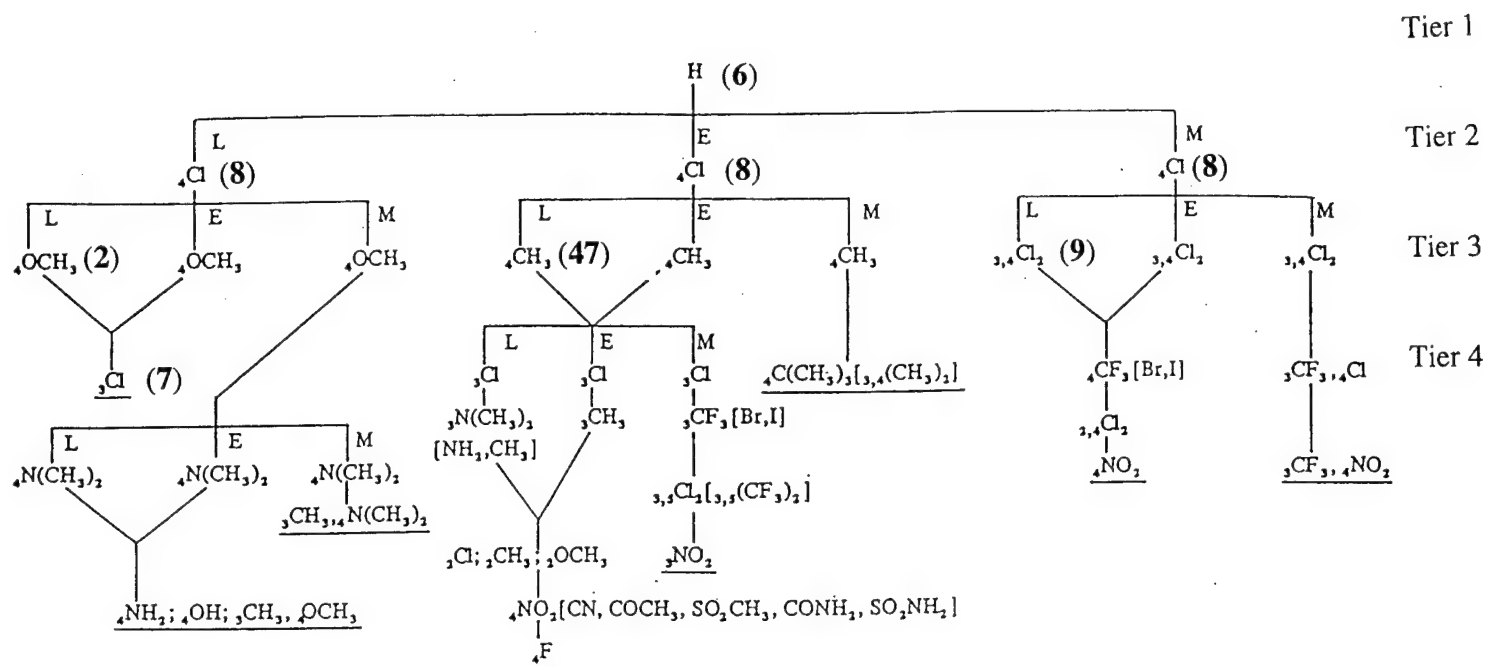
**Scheme 8**



epoxidants tried:

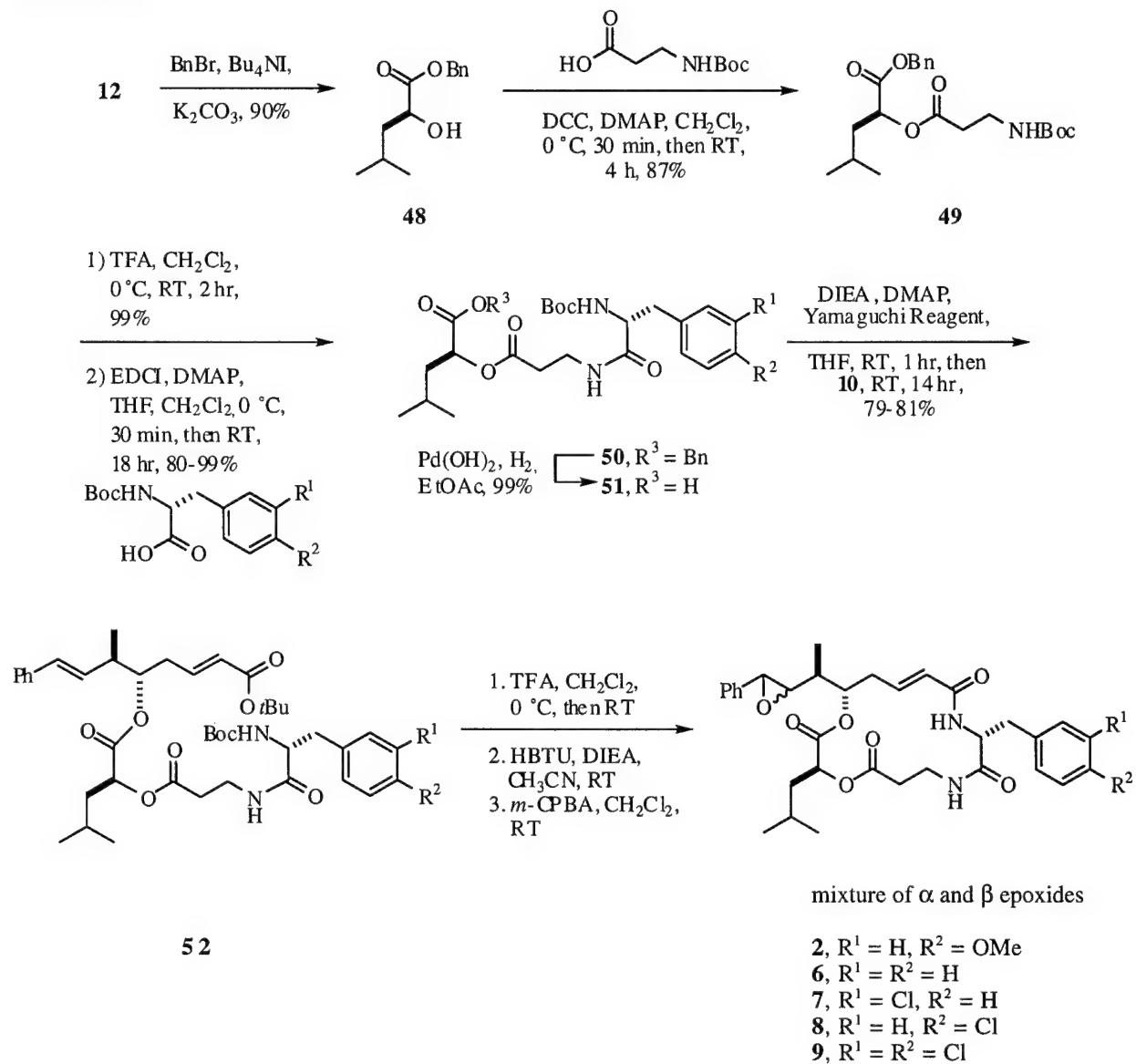


Scheme 9



M=more active, E = equiactive, L = less active compared to the 4-H compound. Descending lines indicate the sequence. Square brackets indicate alternatives. (taken from *Journal of Medicinal Chemistry*, 1972, 15, 1006.)

## Scheme 10



## Appendix Part D - Table

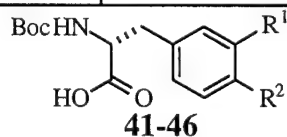
**Table 1.** *In Vitro* tubulin assay and breast cancer cell line cytotoxicity results for cryptophycin A (**1**) and the C3 analogues **4** and **5** and C10 analogues **6-9**.

Assay	Crypto. A	C3 analogues		C10 analogues			
	<b>1</b>	<b>4</b>	<b>5*</b>	<b>6*</b>	<b>7*</b>	<b>8*</b>	<b>9*</b>
IC <sub>50</sub> tubulin polymerization	1.0 $\mu$ M	TBD	5.0 $\mu$ M	>20 $\mu$ M	0.5 pM	>20 $\mu$ M	>20 $\mu$ M
ED <sub>50</sub> MCF-7 breast cancer cell line	13 pM ++++	TBD	80 nM +++	nM +	20.5 pM ++++	nM +	100-600 pM +++
ED <sub>50</sub> MCF-7/ADR breast cancer cell line	50 pM ++++	TBD	1000 nM +++	nM +	115 pM +++	nM +	100-600 pM +++

\*Tested as a mixture of  $\alpha$  and  $\beta$  epoxides. The tubulin polymerization assay,<sup>4</sup> which used tubulin isolated from bovine brain, was conducted by incubating varying concentrations of the analogue of interest with tubulin in PEM buffer containing 0.5 mM GTP and 8% DMSO. After sedimentation, the increase in the apparent absorbance at 350 nM of the unpolymerized protein in the supernatant was recorded. TBD = to be determined.

**Table 2.** Relationship between the C10 building blocks (**41-45**) defined by the Topliss-Martin operational scheme and the C10 analogues **6-9**.

Entry	Tier in the Topliss-Martin Scheme	Substitution pattern	C10 Building Block	Commercially Available	Analogue
1	Tier 1	R <sup>1</sup> = H R <sup>2</sup> = H	<b>41</b>	Yes	<b>6</b>
2	Tier 2	R <sup>1</sup> = H R <sup>2</sup> = Cl	<b>42</b>	Yes	<b>8</b>
3	Tier 3	R <sup>1</sup> = H R <sup>2</sup> = OMe	<b>43</b>	No	<b>2</b>
4	Tier 3	R <sup>1</sup> = H R <sup>2</sup> = Me	<b>44</b>	Yes	<b>47</b> not prepared
5	Tier 3	R <sup>1</sup> = Cl R <sup>2</sup> = Cl	<b>45</b>	Yes	<b>9</b>
6	Tier 4	R <sup>1</sup> = Cl R <sup>2</sup> = H	<b>46</b>	Yes	<b>7</b>



**Appendix**  
**Part E – List of Personnel**

Suzanne B. Buck

**Appendix**  
**Part F – Curriculum Vitae – Suzanne Buck**

**EDUCATION:**

**Ph.D. Candidate, Medicinal Chemistry, Anticipated February 2002**

Department of Medicinal Chemistry, University of Kansas, Lawrence, KS  
Advisor: Professor Gunda I. Georg

**M.S., Medicinal Chemistry, August 1999**

Department of Medicinal Chemistry, University of Kansas, Lawrence, KS

**B.S., Biochemistry, Summa Cum Laude, May 1996**

University of Hartford, West Hartford, CT

**PROFESSIONAL EXPERIENCE:**

**Graduate Research Assistant, July 1996 to present, Medicinal Chemistry**

Department of Medicinal Chemistry, University of Kansas, Lawrence, KS  
Advisor: Dr. Gunda I. Georg

1. Synthetic Organic Chemistry: Cryptophycin chemistry, peptide coupling methods, photochemistry, asymmetric epoxidation, asymmetric synthesis
2. Total Synthesis of Natural Products: Synthesis of Cryptophycin A Analogues

**Undergraduate Research Assistant, Fall 1995 and Spring 1996, Biology**

Department of Biology, University of Hartford, West Hartford, CT  
Advisor: Dr. Martin Cohen

1. Growth and propagation of selected fungi
2. UV-VIS spectrophotometry to test fungi for their ability to solubilize Rhenish Brown coal

**Undergraduate Research Assistant, Summer 1995, Chemistry**

Undergraduate Research Summer Institute  
Department of Chemistry, Vassar College, Poughkeepsie, NY  
Advisor: Dr. Curt W. Beck  
Analysis of fossilized resin using GC-MS, FT-IR

**Undergraduate Research Assistant, Fall 1994 and Spring 1995, Chemistry**

Department of Chemistry, University of Hartford, West Hartford, CT  
Advisor: Dr. Malcolm Stevens  
Synthesis of head-to-head poly(acrylic acid)

**Undergraduate Research Assistant, Summer 1994, Chemistry**

Undergraduate Research Summer Institute  
Department of Chemistry, Vassar College, Poughkeepsie, NY  
Advisor: Dr. Curt W. Beck  
Analysis of Beechwood tar extracts using GC-MS

AWARDS AND HONORS:

**Department of Defense, Breast Cancer Research Program, Predoctoral Fellowship**, Research Training Grant, April 1998 to May 2001  
**National Institutes of Health, Medicinal Chemistry and Pharmacology Training Program, Predoctoral Fellowship**, Research Training Grant, University of Kansas, September 1997 to March 1998  
**Department of Medicinal Chemistry**, University of Kansas, Research Assistantship, July 1996 to August 1997  
**Outstanding Senior Student in Chemistry**, American Institute of Chemists Foundation and New England Institute of Chemists Student Award Certificate for 1996

PROFESSIONAL AFFILIATIONS:

**American Chemical Society**  
Division of Organic Chemistry  
Division of Medicinal Chemistry  
**Sigma Xi, The Scientific Research Society**  
**Toastmasters International**

PUBLICATIONS:

Eggen, M.; Mossman, C. J.; Buck, S. B.; Nair, S. K.; Bhat, L.; Ali, S. M.; Reiff, E. A.; Boge, T. C.; Georg, G. I. Total Synthesis of Cryptophycin-24 (Arenastatin A) Amenable to Structural Modifications in the C16 Side Chain. *J. Org. Chem.* **2000**, *65*, 7792-7799.

Buck, S. B.; Victory, S. F.; Himes, R. H.; Georg, G. I. Epothilones, A New Class of Microtubule-Stabilizing Agents with a Taxol-like Mechanism of Action. *Chemtracts* **1998**, *11*, 671-677.

Beck, C. W.; Stout, E. C.; Hanna, S. Amber of the European Alps *Proceedings of the XIII. International Congress of Prehistoric and Protohistoric Sciences*, Forli, Italy, 4-14 September 1996 (publ. 1998), Vol. 6, Part I, pp. 497-506.

Beck, C. W.; Stout, E. C.; Buck, S. B. Beech Wood Tar, *manuscript in preparation*.

PRESENTATIONS:

Buck, Suzanne B.; Georg, Gunda I. "Total Synthesis and Biological Evaluation of C3 and C10 Analogues of Cryptophycin A." 2001 Winter Conference, Medicinal and Bioorganic Chemistry Foundation, Steamboat Springs, CO, January 28 - February 2, 2001.

Buck, Suzanne B.; Georg, Gunda I. "Studies Towards the Total Synthesis of Cryptophycin A Analogues." 27<sup>th</sup> National Medicinal Chemistry Symposium, Kansas City, MO, June 13 - 17, 2000.

Buck, Suzanne B.; Georg, Gunda I. "Synthesis of C3 Epimer Analogues of Cryptophycin A." Era of Hope, Department of Defense Breast Cancer Research Meeting, Atlanta, Georgia, June 8 - 12, 2000.

Buck, Suzanne B.; Georg, Gunda I. "Studies Towards the Total Synthesis of Cryptophycin A and Analogues." 37<sup>th</sup> MIKI Medicinal Chemistry Meeting, Lawrence, Kansas, April 16-18, 1999.

Hanna, Suzanne B.; Eggen, MariJean; Mossman, Craig J.; Reiff, Emily A.; Boge, Thomas C.; Georg, Gunda I. "Studies Towards the Total Synthesis of Cryptophycin A and Analogues." 36<sup>th</sup> MIKI Medicinal Chemistry Meeting, Chicago, Illinois, May 22-24, 1998.

Eggen, MariJean; Hanna, Suzanne B.; Mossman, Craig J.; Boge, Thomas C.; Reiff, Emily A.; Georg, Gunda I. "Synthesis of Cryptophycins: Use of Acylated Lactams as Reactive Intermediates." *Abstracts of Papers*, 215<sup>th</sup> National Meeting of the American Chemical Society, Dallas, Texas; American Chemical Society, Washington, DC, 1998; ORG 341.

OTHER PROFESSIONAL ACTIVITIES:

**Co-Chairman of the 37<sup>th</sup> MIKI Medicinal Chemistry Meeting**, Lawrence, Kansas, April 16-18, 1999.

**President, Toastmasters International**, University of Kansas Chapter, Fall 1999 and Spring 2000.

VOLUNTEER ACTIVITIES:

**Room Coordinator at the "Carnival of Chemistry,"** sponsored by the University of Kansas Section of the American Chemical Society, National Chemistry Week, 1996, 1997, 1998, 1999.

**Judge for the Douglas County Science Fair**, Lawrence, Kansas, 1997, 1998, 1999.

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# **Total Synthesis of Cryptophycin-24 (Arenastatin A) Amenable to Structural Modifications in the C16 Side Chain**

---

**MariJean Eggen, Craig J. Mossman, Suzanne B. Buck,  
Sajiv K. Nair, Laxminarayan Bhat, Syed M. Ali, Emily A. Reiff,  
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Department of Medicinal Chemistry, University of Kansas,  
Lawrence, Kansas 66045

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# Total Synthesis of Cryptophycin-24 (Arenastatin A) Amenable to Structural Modifications in the C16 Side Chain

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Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045

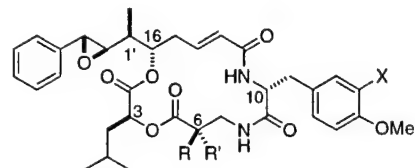
georg@ukans.edu

Received May 19, 2000 (Revised Manuscript Received September 8, 2000)

Two efficient protocols for the synthesis of *tert*-butyl (5*S*,6*R*,2*E*,7*E*)-5-[(*tert*-butyldimethylsilyl)oxy]-6-methyl-8-phenyl-2,7-octadienoate, a major component of the cryptophycins, are reported. The first utilized the Noyori reduction and Frater alkylation of methyl 5-benzyloxy-3-oxopentanoate to set two stereogenic centers, which became the C16 hydroxyl and C1' methyl of the cryptophycins. The second approach started from 3-*p*-methoxybenzyloxypropanal and a crotyl borane reagent derived from (–)- $\alpha$ -pinene to set both stereocenters in a single step and provided the dephenyl analogue, *tert*-butyl (5*S*,6*R*,2*E*)-5-[(*tert*-butyldimethylsilyl)oxy]-6-methyl-2,7-octadienoate, in five steps. This compound was readily converted to the 8-phenyl compound via Heck coupling. The silyloxy esters were efficiently deprotected and coupled to the C2–C10 amino acid fragment to provide desepoxyarenastatin A and its dephenyl analogue. The terminal olefin of the latter was further elaborated via Heck coupling. Epoxidation provided cryptophycin-24 (arenastatin A).

## Introduction

A novel, cyclic depsipeptide isolated from the blue-green algae (cyanobacterium) *Nostoc* sp. ATCC 53789 was reported by Schwartz and co-workers in 1990.<sup>1</sup> This compound demonstrated extremely potent activity against filamentous fungi of the genus *Cryptococcus* and thus was named cryptophycin A (1) (also known as cryptophycin-1, Figure 1).<sup>1</sup> Subsequently, Moore and co-workers isolated the same compound and several structural relatives from *Nostoc* sp. GSV 224.<sup>2,3</sup> Cryptophycin-1 (1), the most abundant of the macrolides, was found to have significant tumor-selective cytotoxicity<sup>3–5</sup> and was not an effective substrate for the P-glycoprotein efflux mechanism in multiple-drug resistant cells.<sup>6</sup> When administered intravenously, depsipeptide 1 was also very effective against mammary, colon, and pancreatic adenocarcinomas in mouse xenographs.<sup>7</sup> Concurrently, Kobayashi and Kitagawa isolated a related cytotoxic agent from the Okinawan marine sponge *Dysidea arenaria* and named it arenastatin A (2).<sup>8–10</sup> It was found that cryptophycin-24 was identical to this compound. Thus far, 25 compounds of the cryptophycin family have been reported through isolation.<sup>2,7</sup>



Cryptophycin-1 R = Me, R' = H, X = Cl (1)  
Cryptophycin-24 (Arenastatin A) R, R' = H, X = H (2)  
Cryptophycin-52 R, R' = Me, X = Cl (3)

Figure 1. Structures of the cryptophycins.

Cryptophycin-1 (1) blocks the cell cycle at the G<sub>2</sub>/M phase apparently through inhibition of tubulin polymerization into microtubules.<sup>11,12</sup> This compound binds to a tubulin site distinct from the colchicine site, but one that may overlap with the vinblastine site.<sup>11,13–15</sup> Its extreme potency (100–1000-fold greater than paclitaxel and vinblastine) has led to additional studies investigating other possible modes of action.<sup>12,16,17</sup> It was found that cryptophycin-1 is a highly potent stabilizer of microtubule dynamics at concentrations ( $\leq 100$  nM) that have no effect on net microtubule polymerization.<sup>16</sup>

Semisynthetic analogues of cryptophycin-1 (1) primarily focusing on the reactive epoxide moiety have resulted in loss of activity except for the halohydrins.<sup>18</sup> In particular, the chlorohydrin derived from cryptophycin-1 (1)

\* To whom correspondence should be addressed. Phone: (785) 864-4498. Fax: (785) 864-5836.

(1) Schwartz, R. E.; Hirsch, C. F.; Sesin, D. F.; Flor, J. E.; Chartrain, M.; Fromling, R. E.; Harris, G. H.; Salvatore, M. J.; Liesch, J. M.; Yudin, K. *J. Ind. Microbiol.* **1990**, *5*, 113.

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demonstrated higher activity in vivo than the parent compound. However, comparisons of tubulin assembly and cell toxicity data indicated that the halohydrins have reduced or no biological activity and that the observed activity resulted from the regeneration of the parent compound **1**.<sup>19</sup> In pursuit of stable analogues, synthetic approaches were developed probing the substituents and stereochemistry of the tyrosine<sup>20</sup> and  $\beta$ -amino acid subunits,<sup>21–23</sup> the stereochemical and electronic effects of the octadienoate ester subunit,<sup>24–26</sup> and replacement of the  $\alpha$ -hydroxy acid subunit with an  $\alpha$ -amino acid.<sup>27</sup> These investigations gave rise to cryptophycin-52 (**3**), a synthetic analogue currently in phase II clinical trials.<sup>28</sup> This analogue is hydrolytically more stable than **1** due to the presence of *gem*-dimethyl substituents on the  $\beta$ -amino acid moiety and demonstrates similar or improved bioactivity. Cryptophycin-52 (**3**) has been shown to stabilize microtubule dynamics<sup>28</sup> and be very effective against numerous human tumor cell lines.<sup>29</sup> This compound also accumulated within cells to a concentration consistent with mitotic arrest without altering microtubule polymer concentration.<sup>30</sup> Paclitaxel, which has been shown to have a second mechanism of action by the hyperphosphorylation of Bcl2, renders cancer cells susceptible to apoptosis.<sup>31</sup> Cryptophycin-52 (**3**), likewise, was recently reported to also have this mechanism of action and to be the most potent agent known in this respect.<sup>32</sup>

The outstanding activity, extreme potency, and arrival of a clinical candidate of the cryptophycins has generated a large amount of interest in the total synthesis of arenastatin A (**2**),<sup>33–36</sup> cryptophycin-1 (**1**),<sup>26,37–40</sup> cryptophycin-2,<sup>41,42</sup> and cryptophycin-52 (**3**),<sup>40,43</sup> as well as the synthesis of the octadienoate ester fragment.<sup>44–48</sup> The following discussion fully discloses our previously reported fragment syntheses,<sup>44,45</sup> as well as the completion of the synthesis of cryptophycin-24 (arenastatin A, **2**).

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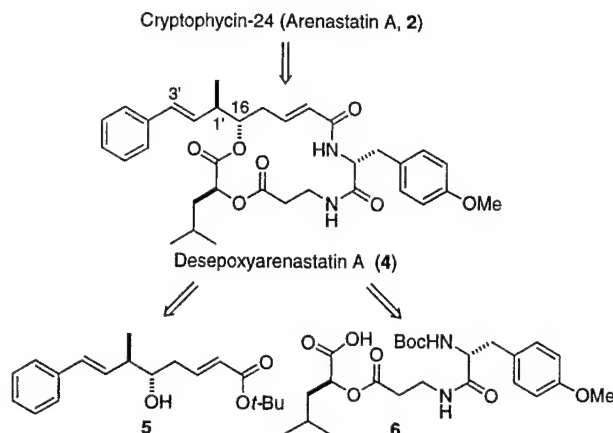
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## Scheme 1



## Results and Discussion

Our interest in structurally flexible fragment syntheses was primarily for structure–activity relationship (SAR) studies, biochemical studies,<sup>11</sup> and photolabeling experiments with tubulin protein. To achieve this goal, we targeted cryptophycin-24 (**2**), which lacks a chiral center at C6 and the chlorine substituent in the tyrosine moiety. The activity of **2**, though slightly diminished in certain cancer cell lines in comparison to cryptophycin-1 (**1**), is similar with respect to microtubule depolymerization.<sup>10,13</sup> The retrosynthetic analysis of cryptophycin-24 (**2**) is shown in Scheme 1. The synthesis of **2** can be simplified by utilizing desepoxyarenastatin **4** as the final precursor with epoxidation being the last step in the total synthesis. Further retrosynthetic analysis of desepoxyarenastatin reveals that **4** can be assembled from ester **5** and amino acid **6**, synthesized from L-leucic acid,  $\beta$ -alanine and D-O-methyltyrosine.

The structurally more complex fragment, ester **5**, was targeted via two approaches (Scheme 2). The first approach, which provided flexibility at the C1' position, utilized an asymmetric reduction of **8** to set the C16 hydroxyl group and then incorporated the second stereocenter anti using a Frater alkylation to provide intermediate **7**. This alkylation strategy allowed potential incorporation of alternative substitution at C1' through

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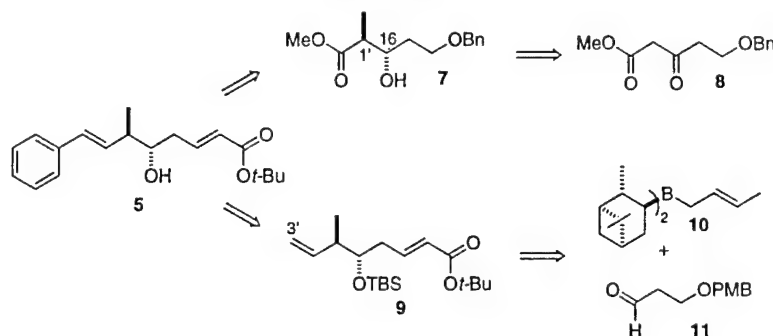
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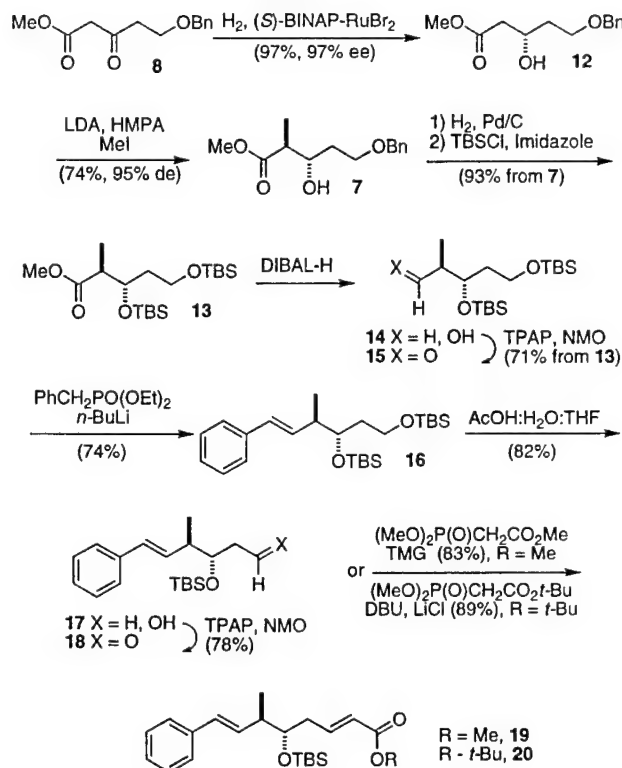
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Scheme 2



Scheme 3



the use of electrophiles other than iodomethane. A second route, in which a dephenyl ester **9** was the targeted synthon, allowed the probing of the C3' aromatic region of the side chain. Ester **9** was derived from a crotyl boration of aldehyde **11** with borane **10**, which incorporated both backbone stereocenters in a single step. With **9** in hand, we could vary the aryl substituents using a Heck coupling strategy in the final step of the fragment synthesis or potentially after the 16-membered cryptophycin macrocycle was intact for thorough SAR studies.

Our first approach (Scheme 3) to the octadienoate ester **5** targeted flexibility in the C1' position in the target compounds. The asymmetric Noyori catalytic hydrogenation<sup>49</sup> of  $\beta$ -keto ester **8**<sup>50</sup> with the (*R*)-Ru(BINAP)Br<sub>2</sub> complex<sup>51</sup> provided (*S*)-hydroxy ester **12**<sup>52</sup> in 97% yield

and 97% ee (enantiomeric excess was determined through chiral HPLC: Daicel Chiralcel OD-H). Frater alkylation<sup>53,54</sup> of the dianion of  $\beta$ -hydroxyester **12** with iodomethane gave anti product **7** in 75% yield and 95% diastereomeric excess. The minor isomer was easily removed by flash chromatography. Debenzylation of **7** (95%), followed by silylation of the resulting diol, provided compound **13** in 98% yield. DIBAL-H reduction of **13** to the corresponding aldehyde **15** and subsequent Horner-Emmons reaction (diethyl benzylphosphonate) yielded styrene **16**. It was found, however, that complete reduction to the primary alcohol **14** with DIBAL-H, followed by TPAP oxidation<sup>55</sup> was experimentally more convenient and provided a comparable overall yield. Selective cleavage of the primary TBS-ether of **16** (HOAc, H<sub>2</sub>O, THF; 1:1:2)<sup>56</sup> was followed by TPAP oxidation to yield aldehyde **18**. The aldehyde **18** was converted directly to the methyl ester **19** in 83% yield or the *tert*-butyl ester **20** in 89% yield by Horner-Emmons homologation with the corresponding phosphonate and tetramethylguanidine or DBU and LiCl,<sup>57</sup> respectively. A comparison of the optical rotation of the methyl ester thus obtained ( $[\alpha]_D = +66.8$ ), with the published data for **19** ( $[\alpha]_D = +68.2$ )<sup>37</sup> verified the high optical purity and correct absolute stereochemistry of our product.

This approach provided the octadienoate ester backbone **20** in 10 steps (overall yield of 20%) from readily available starting materials. The synthesis can be carried out on a relatively large scale. Although this route provided an effective method for modification at C1', we required a method to more efficiently modify the C3' aromatic substituent.

A second approach (Scheme 4) to octadienoate ester **5**, amenable to SAR studies at the C3' phenyl group of the C16 side chain, was achieved starting from aldehyde **11**.<sup>58</sup> The key step utilized the crotyl boration of **11** with crotyl diisopinocampheylborane **10** (prepared from (+)-*B*-methoxydiisopinocampheylborane) to generate the desired stereochemistry at the two chiral centers of **21** in 76% yield (91% ee).<sup>59</sup> Silyl protection of the secondary alcohol with *tert*-butyldimethylsilyl trifluoromethanesulfonate

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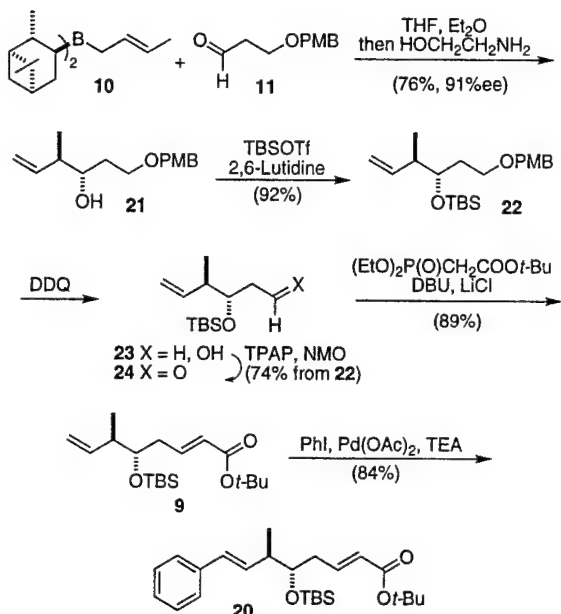
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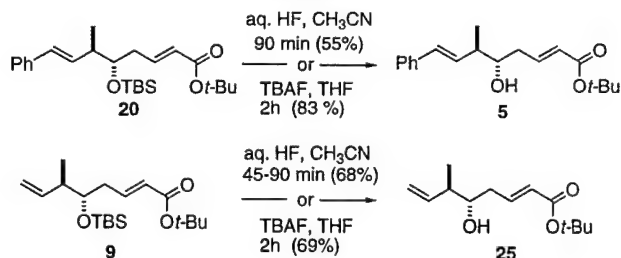
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## Scheme 4



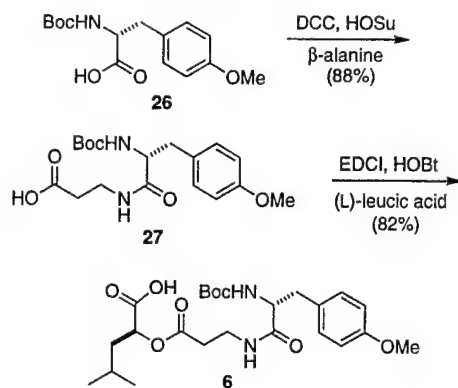
## Scheme 5



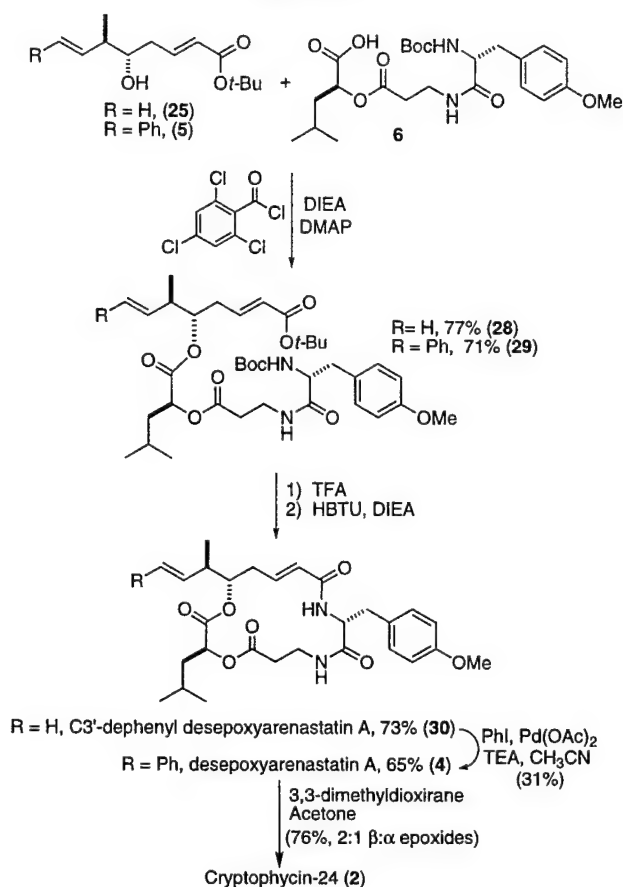
(TBSOTf) in the presence of 2,6-lutidine proceeded at low temperature to silyl ether **22** in 92% yield. Short reaction times were essential for optimal outcome. Rapid deprotection of the *p*-methoxybenzyl ether with DDQ provided a separable mixture of *p*-methoxybenzaldehyde and the desired primary alcohol. However, carrying forward a mixture of *p*-methoxybenzaldehyde and the alcohol **23** was experimentally more convenient. This mixture was subjected to catalytic TPAP oxidation<sup>55</sup> conditions in the presence of NMO producing the easily separable aldehyde **24** after chromatography in 74% yield over two steps. The Horner-Emmons homologation to form the  $\alpha,\beta$ -unsaturated *tert*-butyl ester **9** proceeded cleanly using *tert*-butyl diethylphosphonoacetate, DBU and LiCl.<sup>57</sup> The terminal olefin of compound **9** is the key moiety necessary for modification at what becomes the C3'-aromatic position. Heck coupling<sup>60,61</sup> utilizing Pd(OAc)<sub>2</sub>, PhI and triethylamine provided the aryl synthon **20** in 84% yield (39% overall in 6 steps from aldehyde **11**). Comparison of the optical rotations of this **20** with the rotation of **20** from the Noyori reduction/Frater alkylation route confirmed its optical purity and correct absolute stereochemistry.

Deprotection (Scheme 5) of aryl synthon **20** with 49% HF in acetonitrile provided hydroxy ester **5** in reasonable yield. These conditions were also used for the deprotection of **9**, providing hydroxy ester **25**. Alternatively, it was demonstrated that TBAF deprotection of silyl ethers

## Scheme 6



## Scheme 7



**20** and **9** with a polymer resin workup as described by Parlow and co-workers<sup>62</sup> provided the desired hydroxy esters **5** and **25** cleanly.

The second key synthon **6** was readily synthesized starting from *N*-Boc amino acid **26** (Scheme 6). Activation of **26** with DCC and *N*-hydroxysuccinimide (HOSu) followed by addition of  $\beta$ -alanine provided acid **27**. EDCI activation and subsequent addition of L-leucic acid provided acid **6** in two steps without the necessity of extensive protecting group chemistry.

With the key synthons, hydroxy esters **5** and **25** and acid **6**, in hand, we proceeded with macrocycle formation. Acid **6** was activated (Scheme 7) in a Yamaguchi coupling reaction<sup>63</sup> with 2,4,6-trichlorobenzoyl chloride in the presence of diisopropylethylamine (DIEA) and catalytic

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DMAP. Addition of the alcohols **25** or **5** to the mixed anhydride provided advanced intermediates **28** and **29**, respectively. Deprotection of the *tert*-butyl ester and the *N*-Boc with trifluoroacetic acid (TFA) produced the cyclization precursors. Under dilute conditions, HBTU activation provided the desired macrocycles **30** and **4**. Epoxidation of **4** with dimethyldioxirane<sup>64</sup> using reported conditions<sup>36</sup> completed the total synthesis of cryptophycin-24, arenastatin A (**2**). Through our methodology, we also synthesized the dephenyl desepoxyarenastatin A (**30**) for modification studies on the C16 aromatic side chain. Heck coupling conditions directly converted compound **30** to **4**, albeit in low yield.

In summary, convergent total syntheses of cryptophycin-24 (**2**) amenable to modifications at the C1' and C3' positions of the C16 aromatic side chain were achieved. Introduction of the aryl moiety at the C3' position can be made at a late stage in the synthesis by utilizing dephenyl desepoxyarenastatin A (**30**) or dephenyl synthon **9**. Application of this methodology toward analogues for SAR and biochemical studies exploring the cryptophycin binding site on tubulin are currently in progress.

### Experimental Section

**General Methods.** <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained in CDCl<sub>3</sub> with a 300, 400 or 500 MHz spectrometer. High-resolution mass spectra were obtained at the University of Kansas mass spectrometry support laboratory. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Chiral HPLC analysis was performed using a Chiralcel OD-H column. Column chromatography was carried out on silica gel (230–400 mesh, Merck). THF and diethyl ether were distilled from sodium benzophenone ketyl, CH<sub>2</sub>Cl<sub>2</sub> was freshly distilled from CaH<sub>2</sub>. DIEA, TEA and 2,6-lutidine were distilled from CaH<sub>2</sub> prior to use. All other reagents were commercially available and used without further purification. All moisture-sensitive reactions were carried out under inert atmosphere in oven dried glassware.

**Methyl (3*R*)-5-Benzyloxy-3-hydroxypentanoate (12).** Under a N<sub>2</sub> atmosphere, a mixture of degassed methyl 5-benzyloxy-3-oxopentanoate (0.5 g, 2.12 mmol) and methanol (10 mL) was placed in a Parr hydrogenation bottle. To this was added Ru(BINAP)Br<sub>2</sub> (prepared from bis-(2-methylallyl)cycloocta-1,5-diene ruthenium (II) (4 mg, 0.013 mmol) and (S)-BINAP (8 mg, 0.013 mmol). Hydrogenation was carried out at 50 psi and 50 °C for 5 h. The catalyst was precipitated with Et<sub>2</sub>O (100 mL) and filtered through a plug of Celite. The filtrate was concentrated, and the residue was purified by flash column chromatography (hexane/ether 65:35) to give pure alcohol **12** as an oil (0.49 g, 97%): 97% enantiomeric excess was determined by chiral HPLC (Daicel Chiralcel OD-H, 254 nm, 25:1 hexane/2-propanol, 254 nm, 1 mL/min, retention time **12** = 17.2 min, *ent*-**12** = 21.6 min); [α]<sub>D</sub><sup>20</sup> +11.8° (c 1.07, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.32 (m, 5H), 4.52 (s, 2H), 4.27–4.23 (m, 1H), 3.70 (s, 3H), 3.74–3.62 (m, 2H), 3.41–3.40 (d, *J* = 5.6 Hz, 1H), 2.51–2.50 (d, *J* = 10.5 Hz, 2H), 1.86–1.76 (m, 2H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 172.8, 138.0, 128.5, 128.4, 127.73, 127.68, 73.3, 68.0, 67.0, 51.7, 41.4, 36.0; IR (film) 3500 (br), 1725 cm<sup>-1</sup>; HRMS (FAB, NBA) calcd for C<sub>13</sub>H<sub>19</sub>O<sub>4</sub> (M + H) 239.1283, found 239.1307.

**Methyl (2*S*,3*S*)-5-Benzyloxy-3-hydroxy-2-methylpentanoate (7).** To a solution of diisopropylamine (6.8 mL, 49 mmol) in THF (75 mL) at –78 °C was added *n*-BuLi (2.5 M in hexanes, 17.4 mL, 43.5 mmol). After the mixture was stirred at –78 °C for 20 min, **12** (4.40 g, 18.5 mmol) was added. The reaction mixture was stirred at –78 °C for 20 min and slowly warmed to –20 °C and maintained at this temperature for 90

min. The reaction mixture was again cooled to –78 °C, and a solution of iodomethane (1.73 mL, 27.8 mmol) in HMPA (8.16 mL, 47.0 mmol) was added. The reaction mixture was stirred at –78 °C for 15 min, warmed slowly to –15 °C over 1.5 h, and maintained at this temperature overnight. Following treatment with saturated aqueous NH<sub>4</sub>Cl and extraction with Et<sub>2</sub>O, the organic layer was washed with brine, water and dried (MgSO<sub>4</sub>). The crude product was purified by flash column chromatography (hexane:Et<sub>2</sub>O 3:1) to give product **7** as an oil (3.40 g, 77%): 92% diastereomeric excess was determined by integration of *CHOH* in <sup>1</sup>H NMR; [α]<sub>D</sub><sup>20</sup> +14.0° (c 1.49, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.36–7.26 (m, 5H), 4.52 (s, 2H), 3.94–3.93 (m, 1H), 3.69 (s, 3H), 3.74–3.64 (m, 2H), 3.31–3.30 (d, *J* = 4.7 Hz, 1H), 2.58–2.56 (m, 1H), 1.83–1.72 (m, 2H), 1.20 (d, *J* = 6 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 175.8, 137.8, 128.3 (2C), 127.53, 127.49 (2C), 73.1, 72.0, 68.2, 51.5, 45.4, 33.6, 13.6; IR (film) 3500, 1725 cm<sup>-1</sup>; HRMS (FAB, NBA) calcd for C<sub>14</sub>H<sub>21</sub>O<sub>4</sub> (M + H) 253.1440, found 253.1446.

**Methyl (2*S*,3*S*)-3,5-Bis[(*tert*-butyldimethylsilyloxy)-2-methylpentanoate (13).** To a solution of **7** (3.70 g, 14.68 mmol) in THF (35 mL) was added 10% Pd/C (0.940 g). The solution was flushed with argon and hydrogenated (50 psi) for 3 h. After filtration, solvent was removed under reduced pressure to give pure (by <sup>1</sup>H NMR) diol as an oil. The oil was dissolved in DMF (13 mL) and treated with TBSCl (5.83 g, 38.9 mmol) and imidazole (5.89 g, 77.8 mmol). After 16 h at room temperature, the reaction was treated with saturated aqueous NH<sub>4</sub>Cl (1 mL) and partitioned between Et<sub>2</sub>O and H<sub>2</sub>O. Organic extracts were washed with NaHCO<sub>3</sub>, H<sub>2</sub>O, and brine and dried (Na<sub>2</sub>SO<sub>4</sub>). The crude product was purified by flash column chromatography (hexane/EtOAc 90:10) to give **13** as a clear oil (4.95 g, 93%): [α]<sub>D</sub><sup>20</sup> +14.11° (c 1.885, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 4.14–4.10 (m, 1H), 3.72–3.61 (m, 2H), 3.64 (s, 3H), 2.70–2.66 (m, 1H), 1.69–1.63 (m, 1H), 1.60–1.54 (m, 1H), 1.11–1.09 (d, *J* = 7 Hz, 3H), 0.87 (s, 9H), 0.86 (s, 9H), 0.05 (s, 3H), 0.035 (s, 3H), 0.025 (s, 3H), 0.022 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 175.0, 70.3, 59.2, 51.4, 45.6, 36.0, 25.9 (3C), 25.7 (3C), 18.2, 18.0, 11.4, –4.6, –4.9, –5.4 (2C); IR (film) 1725 cm<sup>-1</sup>; MS (EI) *m/z* 375 (M<sup>+</sup> – Me, 1); HRMS (FAB, TG/G[3:1 thioglycerol/glycerol]) calcd for (M + H) C<sub>19</sub>H<sub>43</sub>O<sub>4</sub>Si<sub>2</sub> 391.2700, found 391.2680.

**(2*R*,3*S*)-3,5-Bis[(*tert*-butyldimethylsilyloxy)-2-methylpentan-1-ol (14).** DIBALH (13.35 mL, 1.0 M in hexanes) was slowly added over a period of 15 min to a solution of ester **13** (2.07 g, 5.31 mmol) at –78 °C in THF (50 mL). The reaction was allowed to warm to –10 °C over 3 h. The reaction was quenched with MeOH (5 mL) and warmed to room temperature after gas evolution ceased. The reaction was partitioned between EtOAc (150 mL) and H<sub>2</sub>O (50 mL). The aqueous layer was extracted with EtOAc. Combined organics were dried (Na<sub>2</sub>SO<sub>4</sub>), concentrated, and subjected to column chromatography (2:1 hexane:Et<sub>2</sub>O) to provide the primary alcohol **14** as a colorless oil (1.74 g, 90%): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.90–3.86 (m, 1H), 3.78–3.75 (dd, *J* = 4, 11 Hz, 1H), 3.66–3.63 (t, *J* = 6.4 Hz, 2H), 3.53–3.49 (dd, *J* = 5.3, 11 Hz, 1H), 1.79–1.74 (m, 3H), 1.01–1.00 (d, *J* = 7 Hz, 3H), 0.88 (s, 9H), 0.87 (s, 9H), 0.075 (s, 3H), 0.070 (s, 3H), 0.03 (s, 6H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 74.1, 65.2, 59.7, 38.6, 37.6, 25.88, 25.86, 18.2, 18.0, 14.3, –4.4, –4.8, –5.3; IR (film) 3440 (br) cm<sup>-1</sup>; HRMS (FAB, NBA/LiOAc) calcd for C<sub>18</sub>H<sub>42</sub>O<sub>3</sub>Si<sub>2</sub>Li (M + Li) 369.2832, found 369.2842.

**(3*R*,4*S*,1*E*)-4,6-Bis[(*tert*-butyldimethylsilyloxy)-3-methyl-6-phenylhexene (16).** Alcohol **14** (200 mg, 0.552 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and CH<sub>3</sub>CN (0.4 mL). TPAP (9 mg, 5 mol %), NMO (97 mg, 0.83 mmol), and 4 Å molecular sieves (115 mg) were added, and the mixture was vigorously stirred for 1 h. The mixture was filtered through Celite. The filtrate was concentrated and purified by flash column chromatography (hexane/EtOAc 95:5) to provide aldehyde **15** (175 mg, 88%). A solution of diethyl benzylphosphonate (219 mg, 0.96 mmol) in THF (4 mL) was cooled to –78 °C. To this was added *n*-BuLi (2.5 M in hexanes, 0.37 mL, 0.91 mmol). The reaction mixture was stirred at –78 °C for 30 min and treated with a solution of aldehyde **15** (175 mg, 0.49 mmol) in THF (1 mL). Stirring was continued at –78 °C for another 1 h, and

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then the reaction was gradually warmed to room temperature over 6 h. The reaction was quenched with  $\text{NH}_4\text{Cl}$  solution and extracted with  $\text{Et}_2\text{O}$ . The combined organic layers were washed with water and brine and dried ( $\text{MgSO}_4$ ). Column chromatography (hexane/ $\text{EtOAc}$  99:1) gave styrene **16** as a colorless oil (155 mg, 74%):  $[\alpha]_D^{20} +23^\circ$  (c 0.77,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.4 (m, 5H), 6.41–6.36 (d,  $J$  = 16 Hz, 1H), 6.23–6.15 (dd,  $J$  = 16, 7.7 Hz, 1H), 3.84 (m, 1H), 3.73–3.65 (m, 2H), 2.49 (m, 1H), 1.70–1.63 (m, 2H), 1.12 (d,  $J$  = 6.9 Hz, 3H), 0.93 (s, 9H), 0.90 (s, 9H), 0.091 (s, 6H), 0.056 (s, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  137.8, 132.8, 129.8, 128.4, 126.9, 126.0, 72.7, 60.1, 42.7, 36.7, 25.9 (6C), 18.3, 18.1, 15.5, -4.5 (2C), -5.3 (2C); IR (film) 3020  $\text{cm}^{-1}$ ; MS (EI)  $m/z$  303 ( $\text{M}^+$  - OTBS, 12); HRMS (FAB, NBA) calcd for  $\text{C}_{25}\text{H}_{46}\text{O}_2\text{Si}_2$  ( $\text{M}^+$ ) 377.2332, found 377.2303.

**(3S,4R,5E)-3-[(tert-Butyldimethylsilyl)oxy]-4-methyl-6-phenyl-5-hexen-1-ol (17).** A solution of **16** (0.123 g, mmol) in  $\text{AcOH}$  and aqueous THF ( $\text{AcOH}/\text{H}_2\text{O}/\text{THF}$  1:1:2, 6 mL) was stirred at room temperature. After 72 h, the reaction mixture was neutralized with saturated  $\text{NaHCO}_3$  solution to pH = 7 and extracted with  $\text{EtOAc}$ . The combined organic layers were washed with water and dried ( $\text{MgSO}_4$ ). Flash column chromatography (hexane/ $\text{EtOAc}$  90:10) gave pure product as colorless oil (0.071 g, 82%):  $[\alpha]_D^{20} +28.3^\circ$  (c 0.675,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.3 (m, 5H), 6.42–6.37 (d,  $J$  = 16 Hz, 1H), 6.18–6.10 (dd,  $J$  = 16, 7.8), 3.91–3.87 (m, 1H), 3.77–3.73 (m, 2H), 2.62–2.51 (m, 1H), 2.05 (br s, 1H), 1.76–1.72 (m, 2H), 1.12–1.10 (d,  $J$  = 6.8 Hz, 3H), 0.91 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  137.5, 132.5, 130.0, 128.5, 128.4, 127.0, 126.0, 74.6, 60.1, 42.7, 35.0, 25.9, 18.0, 14.8, -4.3, -4.6; IR (film) 3350 (br), 3020  $\text{cm}^{-1}$ ; HRMS (FAB, NBA/ $\text{LiOAc}$ ) calcd for ( $\text{M} + \text{Li}$ )  $\text{C}_{19}\text{H}_{32}\text{O}_2\text{SiLi}$  327.2332, found 327.2323.

**(3S,4R,5E)-3-[(tert-Butyldimethylsilyl)oxy]-4-methyl-6-phenyl-5-hexenal (18).** To alcohol **17** (65 mg, 0.2 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) and  $\text{CH}_3\text{CN}$  (0.2 mL) were added TPAP (3.5 mg, 5 mol %), NMO (35 mg, 0.3 mmol), and 4 Å molecular sieves (50 mg). The mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through Celite and subjected directly to flash column chromatography (hexane: $\text{Et}_2\text{O}$  95:5 to 90:10) which gave aldehyde **18** as an oil (51 mg, 78%). Aldehyde **18** was used as obtained in the next reaction.

**Methyl (5S,6R,2E,7E)-5-[(tert-Butyldimethylsilyl)oxy]-6-methyl-8-phenyl-2,7-octadienoate (19).** Procedure as previously reported:<sup>37</sup>  $[\alpha]_D^{20} +67^\circ$  (c 0.63,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.2–7.4 (m, 5H), 6.96 (ddd,  $J$  = 15.6, 7.8, 7.5 Hz, 1H), 6.37 (d,  $J$  = 16 Hz, 1H), 6.16 (dd,  $J$  = 15.9, 8.1 Hz, 1H), 5.84 (d,  $J$  = 15.7 Hz, 1H), 3.75 (m, 1H), 3.72 (s, 3H), 2.44 (m, 1H), 2.36 (m, 2H), 1.10 (d,  $J$  = 6.9 Hz, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  166.8, 146.4, 137.6, 131.9, 130.4, 128.5, 127.0, 126.0, 122.9, 75.0, 51.4, 42.8, 37.6, 25.8, 18.1, 16.2, -4.4, -4.5; IR (film) 2950, 1720, 1650  $\text{cm}^{-1}$ ; MS (EI)  $m/z$  374 ( $\text{M}^+$ , 1); HRMS (FAB, NBA) calcd for  $\text{C}_{22}\text{H}_{35}\text{O}_3\text{Si}$  ( $\text{M} + \text{H}$ ) 375.2355, found 375.2367.

**tert-Butyl (5S,6R,2E,7E)-5-[(tert-Butyldimethylsilyl)oxy]-6-methyl-8-phenyl-2,7-octadienoate (20).** A solution of *tert*-butyldiethylphosphonoacetate (0.864 mL, 3.68 mmol), DBU (0.330 mL, 2.21 mmol), and  $\text{LiCl}$  (0.109 g, 2.58 mmol) was stirred vigorously at room temperature for 30 min in  $\text{CH}_3\text{CN}$  (35 mL) and then added dropwise to a solution of aldehyde **18** (0.586 g, 1.84 mmol) in  $\text{CH}_3\text{CN}$  (4.4 mL). After 75 min, the reaction mixture was washed with saturated aqueous  $\text{NH}_4\text{Cl}$  solution,  $\text{H}_2\text{O}$ , and brine. The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. Flash column chromatography (hexane/ $\text{EtOAc}$ , 95:5) provided *tert*-butyl ester **20** as an oil (0.582 g, 76%):  $[\alpha]_D^{20} +69^\circ$  (c 0.73,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.37–7.17 (m, 6H), 6.86–6.76 (dt,  $J$  = 8, 16 Hz, 1H), 6.38–6.33 (d,  $J$  = 16 Hz, 1H), 6.18–6.10 (dd,  $J$  = 8, 16 Hz, 1H), 5.74–5.69 (d,  $J$  = 16 Hz, 1H), 3.74–3.69 (app q,  $J$  = 6 Hz, 1H), 2.47–2.40 (m, 1H), 2.32–2.27 (m, 2H), 1.45 (s, 9H), 1.09–1.06 (d,  $J$  = 7 Hz, 3H), 0.88 (s, 9H), 0.033 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  165.8, 144.8, 137.6, 132.0, 130.4, 128.5 (2 C), 127.0, 126.0 (2 C), 125.1, 80.0, 75.1, 42.8, 37.3, 28.1 (3 C), 18.1, 16.1, -4.4, -4.5; IR (film) 2910, 1695, 1645  $\text{cm}^{-1}$ ; MS (CI)  $m/z$  417 ( $\text{M} + 1$ ); HRMS (FAB, NBA) calcd for  $\text{C}_{25}\text{H}_{41}\text{O}_3\text{Si}$  ( $\text{M} + \text{H}$ ) 417.2825, found 417.2848.

**(3S,4R)-1-(4-Methoxybenzyloxy)-4-methyl-5-hexen-3-ol (21).** To a stirred mixture of potassium *tert*-butoxide (7.75 g, 69.06 mmol), THF (72 mL), and *trans*-2-butene (25.7 mL, 89.8 mmol) was added *n*-BuLi (1.6 M in hexanes, 43.2 mL, 69.1 mmol) at  $-78^\circ\text{C}$ . After the addition was complete, the mixture was stirred at  $-45^\circ\text{C}$  for 10 min, producing a bright canary yellow/orange solution. This solution was recooled to  $-78^\circ\text{C}$ , and to it was added dropwise a solution of (+)-*B*-methoxydiisopinocampheylborane (26.23 g, 82.87 mmol) in  $\text{Et}_2\text{O}$  (89 mL). After the reaction mixture was stirred at  $-78^\circ\text{C}$  for 30 min,  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (11.74 mL, 92.54 mmol) was added dropwise followed immediately by aldehyde **11**<sup>58</sup> (16.11 g, 82.87 mmol). The reaction was stirred for an additional 3 h at  $-78^\circ\text{C}$ . The mixture was then concentrated, redissolved in dry  $\text{Et}_2\text{O}$  (150 mL), and cooled to  $0^\circ\text{C}$ , and ethanolamine (4.2 mL) was added with vigorous stirring. The reaction was warmed to room temperature and stirred for 72 h. The mixture was then filtered and the filtrate concentrated. Column chromatography of the filtrate (95:5 to 80:20 hexane/ $\text{EtOAc}$ ) provided the pure alcohol **21** (13.20 g, 76%): 91% enantiomeric excess was determined by chiral HPLC comparing alcohol **21** with alcohol *ent*-**21** derived from reaction with the (–)-borane (Daicel Chiralcel OD-H, 254 nm, 99:1 hexanes/2-propanol, 0.5 mL/min, retention time: *ent*-**21** = 32.2 min, **21** = 34.6 min);  $[\alpha]_D^{20} +2.7^\circ$  (c 1.5,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.28–7.26 (d,  $J$  = 8.7 Hz, 2H), 6.91–6.88 (d,  $J$  = 8.7 Hz, 2H), 5.87–5.78 (m, 1H), 5.11 (br s, 1H), 5.08–5.06 (br d,  $J$  = 5 Hz, 2H), 4.47 (s, 2H), 3.82 (s, 3H), 3.74–3.61 (m, 3H), 2.82–2.81 (d,  $J$  = 3 Hz, 1H), 2.28–2.22 (m, 1H), 1.76–1.69 (m, 2H), 1.07–1.05 (d,  $J$  = 6.9 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  159.2, 140.5, 130.1, 129.3 (2C), 115.4, 113.8 (2C), 74.3, 73.0, 68.9, 55.3, 44.0, 33.5, 15.8; IR (film) 3420, 3040  $\text{cm}^{-1}$ ; HRMS (FAB, NBA) calcd for  $\text{C}_{15}\text{H}_{22}\text{O}_3$  ( $\text{M}^+$ ) 250.1569, found 250.1577.

**(3S,4R)-3-[(tert-Butyldimethylsilyl)oxy]-1-(4-methoxybenzyloxy)-4-methyl-5-hexene (22).** Alcohol **21** (0.150 g, 0.599 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (6 mL) and cooled to  $-78^\circ\text{C}$ . 2,6-Lutidine (0.14 mL, 1.20 mmol) was added, followed by TBSOTf (0.165 mL, 0.719 mmol) dropwise via syringe. After 10 min, the reaction was quenched with saturated aqueous  $\text{NaHCO}_3$ . The aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$ . The combined organics were dried ( $\text{MgSO}_4$ ), filtered, and concentrated. Flash column chromatography (90:10 hexane/ $\text{EtOAc}$ ) provided a colorless oil (0.201 g, 92%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.25–7.23 (d,  $J$  = 8.6 Hz, 2H), 6.88–6.86 (d,  $J$  = 8.6 Hz, 2H), 5.80–5.71 (ddd,  $J$  = 7, 11, 16 Hz, 1H), 5.00 (br s, 1H), 4.97–4.95 (br d,  $J$  = 7 Hz, 1H), 4.44–4.41 (d,  $J$  = 11 Hz, 1H), 4.39–4.36 (d,  $J$  = 11 Hz, 1H), 3.80 (s, 3H), 3.77–3.73 (m, 1H), 3.48–3.45 (dt,  $J$  = 2, 7 Hz, 2H), 2.29–2.26 (m, 1H), 1.70–1.63 (m, 2H), 1.00–0.98 (d,  $J$  = 6.9 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  159.1, 140.7, 130.7, 129.2 (2C), 114.5, 113.7 (2C), 72.5, 67.1, 55.3, 43.4, 33.2, 25.9, 18.1, 14.5, -4.4, -4.5; IR (film) 3080  $\text{cm}^{-1}$ ; HRMS (FAB, NBA) calcd for  $\text{C}_{21}\text{H}_{36}\text{O}_3\text{Si}$  ( $\text{M}^+$ ) 364.2434, found 364.2442.

**(3S)-3-[(tert-Butyldimethylsilyl)oxy]-4-methyl-5-hexen-1-ol (23).** The *p*-methoxybenzyl ether **22** (1.600 g, 4.388 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (20.7 mL). Water (1.3 mL) and solid DDQ (0.996 g, 4.388 mmol) were added. After 1 h,  $\text{CH}_2\text{Cl}_2$  (200 mL) and saturated aqueous  $\text{NaHCO}_3$  (40 mL) were added. The organic layer was further washed with  $\text{NaHCO}_3$  and brine until the organics were pale yellow in color. The organics were dried ( $\text{MgSO}_4$ ), filtered and concentrated. Flash column chromatography (80:20 hexane/ $\text{EtOAc}$ ) provided a mixture of desired primary alcohol **23** and *p*-methoxybenzaldehyde which was used without further purification in the next reaction:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.78–5.70 (ddd,  $J$  = 7, 10.6, 17 Hz, 1H), 5.04–5.02 (br d,  $J$  = 7 Hz, 1H), 4.99 (br s, 1H), 3.83–3.77 (m, 1H), 3.73–3.69 (m, 2H), 2.39–2.35 (m, 1H), 1.68–1.63 (m, 2H), 1.00–0.99 (d,  $J$  = 6.9 Hz, 3H), 0.89 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  132.0, 114.3, 74.3, 60.5, 43.2, 34.6, 25.8, 18.0, 13.9, -4.4, -4.6.

**(3S)-3-[(tert-Butyldimethylsilyl)oxy]-4-methyl-5-hexenal (24).** To alcohol **23** was added  $\text{CH}_2\text{Cl}_2$  (15 mL) and freshly activated crushed 4 Å molecular sieves (0.380 g), NMO (0.617 g, 5.27 mmol) and TPAP (0.077 g, 0.219 mmol). After the reaction was complete by TLC (15–30 min), the mixture was

concentrated and directly purified by flash chromatography (95:5 hexane/EtOAc) to provide aldehyde **24** as an oil (0.784 g, 74% over two steps):  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  9.78–9.72 (t,  $J$  = 2 Hz, 1H), 5.79–5.70 (ddd,  $J$  = 7, 10.5, 17 Hz, 1H), 5.06–5.04 (m, 1H), 5.05–4.99 (m, 1H), 4.20–4.17 (m, 1H), 2.50–2.43 (app dt,  $J$  = 2, 6 Hz, 2H), 2.39–2.33 (m, 1H), 1.03–1.01 (d,  $J$  = 6.9 Hz, 3H), 0.87 (s, 9H), 0.08 (s, 3H), 0.04 (s, 3H); IR (film) 3090, 1715  $\text{cm}^{-1}$ .

**tert-Butyl (5S,6R,2E)-5-[(tert-Butyldimethylsilyloxy]-6-methyl-2,7-octadienoate (9).** *tert*-Butyldiethylphosphonoacetate (1.16 mL, 4.95 mmol), DBU (0.44 mL, 2.97 mmol), and LiCl (0.147 g, 3.47 mmol) were stirred vigorously at room temperature for 30 min in  $\text{CH}_3\text{CN}$  (10.8 mL). The solution was added dropwise via syringe to a solution of aldehyde **24** (0.600 g, 2.48 mmol) in  $\text{CH}_3\text{CN}$  (5.7 mL). After 2 h, saturated aqueous  $\text{NH}_4\text{Cl}$  solution was added. The organics were further washed with brine. The aqueous layers were cross-extracted with EtOAc. The combined organics were dried ( $\text{MgSO}_4$ ), filtered and concentrated. Flash chromatography (95:5 hexane/EtOAc) provided product as a colorless oil (0.750 g, 89%):  $[\alpha]_D^{20} + 0.7^\circ$  (c 2.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.82–6.75 (dt,  $J$  = 8, 16 Hz, 1H), 5.80–5.71 (buried, 1H), 5.74–5.70 (br d,  $J$  = 16 Hz, 1H), 5.03 (br s, 1H), 5.01–4.99 (br d,  $J$  = 9 Hz, 1H), 3.67–3.63 (m, 1H), 2.29–2.23 (m, 3H), 1.46 (s, 9H), 1.00–0.99 (d,  $J$  = 6.8 Hz, 3H), 0.88 (s, 9H), 0.033 (s, 3H), 0.027 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  165.7, 145.1, 140.1, 125.0, 115.1, 80.0, 74.9, 43.3, 36.8, 28.1 (3 C), 25.8 (3 C), 18.1, 15.3, –4.4, –4.6; IR (film) 3060, 1695  $\text{cm}^{-1}$ ; HRMS (FAB, NBA) calcd for  $\text{C}_{19}\text{H}_{37}\text{O}_5\text{Si}$  (M + H) 341.2512, found 341.2514.

**tert-Butyl (5S,6R,2E,7E)-5-[(tert-Butyldimethylsilyloxy]-6-methyl-8-phenyl-2,7-octadienoate (20).**<sup>36</sup> Olefin **9** (75 mg, 0.22 mmol) was weighed into a dry seal tube and flushed with argon.  $\text{CH}_3\text{CN}$  (2 mL) was added, followed by iodobenzene (49 mg, 0.24 mmol),  $\text{Pd}(\text{OAc})_2$  (2.5 mg, 0.011 mmol), and TEA (0.31 mL, 2.2 mmol). The tube was sealed and heated at 80–85  $^\circ\text{C}$  for 24 h. The mixture was concentrated and subjected directly to column chromatography (90:10 hexane/EtOAc) to provide the product **20** as an oil (77 mg, 83%):  $[\alpha]_D^{20} + 64^\circ$  (c 0.73,  $\text{CHCl}_3$ );  $[\alpha]_D^{20} + 69^\circ$  (c 0.73,  $\text{CHCl}_3$ ) via the Noyori reduction/Frater alkylation route; spectral data consistent with other route.

**tert-Butyl (5S,6R,2E,7E)-5-Hydroxy-6-methyl-8-phenyl-2,7-octadienoate (5).**<sup>36</sup> **HF Procedure.** HF (48% w/w, aq, 13  $\mu\text{L}$ ) was added to ester **20** (0.077 g, 0.19 mmol) dissolved in  $\text{CH}_3\text{CN}$  (1.9 mL) at 0  $^\circ\text{C}$ . After 1.5 h,  $\text{CH}_2\text{Cl}_2$  (10 mL) and saturated aqueous  $\text{NaHCO}_3$  solution were added until the pH was neutral. The aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$ . Combined organics were dried ( $\text{MgSO}_4$ ), filtered, and concentrated. Flash chromatography (90:10 to 85:15 hexane/EtOAc) provided a pale yellow oil (30 mg, 54%).

**TBAF Procedure.** The ester **20** (25 mg, 0.060 mmol) was dissolved in THF (530  $\mu\text{L}$ ). TBAF solution (1 M in THF, 66  $\mu\text{L}$ , 0.066 mmol) was added dropwise. After 2 h, Amberlyst-15 (180 mg), the calcium salt of Amberlyst-15 (180 mg),<sup>62</sup> and 1 mL of THF were added and the mixture stirred for an additional 2.5 h. The resin was removed by filtration and rinsed with  $\text{CH}_2\text{Cl}_2$ . The filtrate was concentrated and subjected to a short plug of silica (80:20 hexane/EtOAc), providing a pale yellow oil (14 mg, 78%):  $[\alpha]_D^{20} + 66^\circ$  (c 0.80,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.37–7.36 (d,  $J$  = 7 Hz, 2H), 7.32–7.28 (app t,  $J$  = 7 Hz, 2H), 7.25–7.20 (t,  $J$  = 7 Hz, 1H), 6.95–6.88 (dt,  $J$  = 7.5, 15.6 Hz, 1H), 6.48–6.44 (d,  $J$  = 16 Hz, 1H), 6.17–6.11 (dd,  $J$  = 8.6, 16 Hz, 1H), 5.86–5.82 (br d,  $J$  = 15.6 Hz, 1H), 3.67–3.62 (m, 1H), 2.48–2.38 (m, 2H), 2.36–2.28 (m, 1H), 1.86 (br s, 1H), 1.47 (s, 9H), 1.15 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  165.7, 144.0, 137.0, 131.8, 130.9, 128.5 (2C), 127.4, 126.1 (2C), 125.4, 80.2, 73.8, 43.2, 37.1, 28.1 (3C), 16.8; IR (film) 3420, 1690, 1635  $\text{cm}^{-1}$ ; MS (CI)  $m/z$  320 (M +  $\text{NH}_3$ , 9), 303 (M + H, 3), 264 (100); HRMS (FAB, NBA) calcd for  $\text{C}_{19}\text{H}_{27}\text{O}_3$  (M + H) 303.1960, found 303.1980.

**tert-Butyl (5S,6R,2E)-5-Hydroxy-6-methyl-2,7-octadienoate (25).** **HF Procedure.** Ester **9** (100 mg, 0.294 mmol) was dissolved in  $\text{CH}_3\text{CN}$  (0.294 mL) and cooled to 0  $^\circ\text{C}$ . HF (48% w/w, aq, 24  $\mu\text{L}$ ) was added. After 25 min, saturated aqueous  $\text{NaHCO}_3$  solution was added. The aqueous layer was

extracted with  $\text{CH}_2\text{Cl}_2$ . Organics were dried ( $\text{MgSO}_4$ ), filtered, and concentrated. Flash column chromatography (90:10 to 85:15 hexane/EtOAc) provided the desired alcohol as a colorless oil (45 mg, 68%).

**TBAF Procedure.** Ester **9** (25 mg, 0.073 mmol) was dissolved in THF (650  $\mu\text{L}$ ). TBAF solution (1 M in THF, 81  $\mu\text{L}$ , 0.081 mmol) was added dropwise. After 2 h, Amberlyst-15 (220 mg), the calcium salt of Amberlyst-15 (220 mg),<sup>62</sup> and 1 mL of THF were added and the mixture stirred for an additional 2.5 h. The resin was removed by filtration and thoroughly rinsed with  $\text{CH}_2\text{Cl}_2$ . The filtrate was concentrated and subjected to a short plug of silica (80:20 hexane/EtOAc) to provide an oil (11 mg, 69%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.93–6.85, 5.85–5.80, 5.78–5.69, 5.15–5.14 (m, 1H), 5.13–5.10 (m, 1H), m (1), dddd (1.5,  $J$  = 4, 6.9, 14 Hz, 1H), m (2H), 1.67–1.66 (d,  $J$  = 4 Hz, 1H), 1.47 (s, 9H), 1.05–1.04 (d,  $J$  = 6.9 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  166.5, 144.0, 139.6, 125.5, 116.9, 80.2, 73.4, 43.8, 36.9, 28.1 (3 C), 16.2; IR (film) 3460, 3100, 1655  $\text{cm}^{-1}$ ; MS (CI)  $m/z$  244 (M +  $\text{NH}_3$ , 15), 227 (M + H, 8), 188 (100); HRMS (FAB, NBA) calcd for  $\text{C}_{13}\text{H}_{23}\text{O}_3$  (M + H) 227.1647, found 227.1669.

**3-[(2R)-(tert-Butoxycarbonylamino)-3-(4-methoxyphenyl)propanoylamino]propanoic Acid (27).** DCC (4.37 g, 21.2 mmol) in DME (25 mL) was added dropwise to a solution of Boc-D-Tyr(Me)OH **26** (5.00 g, 16.9 mmol) and *N*-hydroxysuccinimide (2.92 g, 25.4 mmol) in DME (75 mL) at 0  $^\circ\text{C}$ . The reaction mixture was stirred at 0  $^\circ\text{C}$  for 30 min and at room temperature for 24 h. A solution of 3-aminopropionic acid (2.10 g, 20.3 mmol) and TEA (4.72 mL, 34.0 mmol) in water (50 mL) was slowly added in 10 portions. After 2 h, the reaction mixture was filtered and the residue washed with water. The combined filtrates were concentrated, and a  $\text{K}_2\text{CO}_3$  (aq, 5%) solution was added to the residue until pH 9. The aqueous layer was washed with  $\text{CH}_2\text{Cl}_2$ , acidified to pH 2 with 10% aqueous HCl, and extracted with  $\text{CH}_2\text{Cl}_2$ . This organic phase was then washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated to an oily residue. The residue was triturated with pentane and the resulting precipitate was filtered. The product was recrystallized (ether/pentane) to give pure acid **27** as a crystalline solid (5.50 g, 88%): mp 103–105  $^\circ\text{C}$ ;  $[\alpha]_D^{20} - 7.9^\circ$  (c 1.5,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.06 (d,  $J$  = 8.6 Hz, 2H), 6.98 (br s, 1H), 6.83 (d,  $J$  = 8.6 Hz, 2H), 5.48 (br d,  $J$  = 8.5 Hz, 1H), 4.45 (br d,  $J$  = 7.6 Hz, 1H), 3.73 (s, 3H), 3.47 (br s, 1H), 3.45 (br s, 1H), 2.89 (d,  $J$  = 6.7 Hz, 2H), 2.44 (m, 2H), 1.36 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.3, 171.7, 158.5, 155.8, 130.2(2C), 128.4, 113.9(2C), 80.5, 55.6, 55.1, 38.2, 34.4, 33.5, 28.2(3C); IR (KBr) 3336, 1710, 1684, 1649  $\text{cm}^{-1}$ ; HRMS (FAB, TG/G) calcd for  $\text{C}_{18}\text{H}_{27}\text{N}_2\text{O}_6$  (M + H) 367.1881, found 367.1875.

**(2S)-2-[3-(2R)-2-tert-Butoxycarbonylamino-3-(4-methoxyphenyl)propanoylamino]propanoyloxy]-4-methylpentanoic Acid (6).**<sup>36</sup> To a stirred solution of acid **27** (5.50 g, 15 mmol) and *N*-hydroxysuccinimide (3.40 g, 30 mmol) in DME (100 mL) and  $\text{CH}_2\text{Cl}_2$  (100 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.80 g, 25 mmol) at room temperature. After 24 h, the reaction mixture was diluted with EtOAc (200 mL) and washed with water, 1% HCl, 5% aqueous  $\text{NaHCO}_3$  solution,  $\text{H}_2\text{O}$ , and brine. The organic phase was then dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to give the activated ester. The intermediate was dissolved in  $\text{CH}_3\text{CN}$  (100 mL), and DMAP (9.16 g, 75 mmol) and L-leucic acid (4.95 g, 37.5 mmol) were added at room temperature. The reaction mixture was stirred for 18 h and then acidified with HCl (0.1 N) at 0  $^\circ\text{C}$  until pH 2. The solution was extracted with EtOAc, and the combined extracts were washed with  $\text{H}_2\text{O}$  and brine. The mixture was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated. The residue was purified by column chromatography ( $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  3:1) to give acid **6** (5.90 g, 82%) as a foam:  $[\alpha]_D^{20} - 21^\circ$  (c 0.77,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.05 (d,  $J$  = 8.5 Hz, 2H), 6.85 (br s, 1H), 6.77 (br d,  $J$  = 7.6 Hz, 2H), 5.36 (m, 1H), 5.12 (m, 1H), 4.28 (m, 1H), 3.74 (s, 3H), 3.45 (br s, 2H), 2.95 (br d,  $J$  = 6.2 Hz, 2H), 2.52 (m, 2H), 1.75 (m, 3H), 1.36 (s, 9H), 0.94 (d,  $J$  = 6.2 Hz, 3H), 0.91 (d,  $J$  = 6.2 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.2, 171.7, 158.5, 155.8, 130.3, 128.4, 113.9, 80.5, 71.2, 55.2, 39.4, 37.9, 33.9, 28.2, 24.6, 23.0, 21.5, 14.1;

MS (FAB, TG/G)  $m/z$  481.3 (M + H); HRMS (FAB, TG/G) calcd for  $C_{24}H_{39}N_2O_8$  (M + H) 481.2550, found 481.2573.

**tert-Butyl (5S,6R,2E)-5-[(2S)-2-[3-[(2R)-2-tert-Butoxycarbonylamino-3-(4-methoxyphenyl)propanoylamino]propanoyloxy]-4-methylpentanoyloxy]-6-methylocta-2,7-dienoate (28).** *N*-Boc amino acid **6** (314 mg, 0.65 mmol) was dissolved in THF (12.5 mL). DIEA (142  $\mu$ L, 0.82 mmol), 2,4,6-trichlorobenzoyl chloride (97  $\mu$ L, 0.62 mmol), and DMAP (5 mg) were added. After 30 min, the alcohol **25** (74 mg, 0.33 mmol) in THF (300  $\mu$ L) was added dropwise via syringe. After 45 min, saturated aqueous  $NaHCO_3$  solution was added. The aqueous layer was then extracted with  $CH_2Cl_2$ . The combined organics were dried ( $MgSO_4$ ), filtered, and concentrated. Flash chromatography (80:20 to 70:30 hexanes/EtOAc) provided the desired ester **28** as a thick oil (173 mg, 77%):  $[\alpha]^{20}_D$  -24.6° (c 1.00,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.12–7.10 (d,  $J$  = 8.5 Hz, 2H), 6.80–6.78 (d,  $J$  = 8.5 Hz, 2H), 6.73 (buried m, 1H), 5.81–5.77 (br d,  $J$  = 15.6 Hz, 1H), 5.69–5.60 (ddd,  $J$  = 8, 10.8, 16.6 Hz, 1H), 5.44–5.42 (br d,  $J$  = 8 Hz, 1H), 5.07 (s, 1H), 5.05–5.03 (d,  $J$  = 7 Hz, 1H), 4.98–4.94 (dd,  $J$  = 4, 10 Hz, 1H), 4.96–4.92 (buried, 1H), 4.38–4.32 (m, 1H), 3.74 (s, 3H), 3.61–3.55 (m, 1H), 3.48–3.43 (m, 1H), 3.14–3.09 (dd,  $J$  = 5.5, 14 Hz, 1H), 2.93–2.88 (m, 1H), 2.51–2.38 (m, 5H), 1.78–1.68 (m, 3H), 1.59–1.52 (m, 1H), 1.46 (s, 9H), 1.34 (s, 9H), 1.00–0.98 (d,  $J$  = 7 Hz, 3H), 0.92–0.91 (d,  $J$  = 6.5 Hz, 3H), 0.90–0.88 (d,  $J$  = 6.5 Hz, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  171.3, 170.6, 165.7, 158.3, 155.4, 141.8, 138.3, 130.3 (2C), 129.1, 126.0, 116.6, 113.8, 80.4, 79.5, 76.2, 71.2, 55.9, 55.1, 41.2, 39.6, 37.7, 35.1, 34.6, 34.3, 28.2 (3C), 28.1 (3C), 24.6, 23.0, 21.4, 16.4; MS (FAB, TG/G)  $m/z$  689.4 (M + H); HRMS (FAB, TG/G) calcd for  $C_{37}H_{57}N_2O_{10}$  (M + H) 689.4013, found 689.4030.

**Dephenyldesepoxyarenastatin A (30).** The starting ester **28** (170 mg, 1.48 mmol) was dissolved in  $CH_2Cl_2$  (5 mL), and TFA (300  $\mu$ L) was added. After 1 h, toluene (5 mL) was added and the reaction mixture was concentrated in vacuo. The mixture was then redissolved in  $CH_3CN$  (23 mL), and DIEA (123  $\mu$ L, 0.706 mmol) and HBTU (107 mg, 0.282 mmol) were added. After the mixture was stirred for 1 h, saturated aqueous  $NaHCO_3$  and  $CH_2Cl_2$  were added. The aqueous layer was extracted with  $CH_2Cl_2$ . The combined organics were dried ( $MgSO_4$ ), filtered, and concentrated. Flash chromatography (7:1 to 4:1  $CH_2Cl_2$ /acetone) provided the desired macrocycle **30** as a colorless oil (88 mg, 73%):  $[\alpha]^{20}_D$  +24° (c 0.80,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.11–7.09 (d,  $J$  = 8.6 Hz, 2H), 7.05–7.02 (t,  $J$  = 5.7 Hz, 1H), 6.81–6.79 (d,  $J$  = 8.6 Hz, 2H), 6.71–6.63 (ddd,  $J$  = 5, 10.3, 15 Hz, 1H), 5.84–5.82 (d,  $J$  = 8 Hz, 1H), 5.75–5.71 (d,  $J$  = 14 Hz, 1H), 5.69–5.65 (m, 1H), 5.08 (br s, 1H), 5.05–5.04 (d,  $J$  = 6 Hz, 1H), 5.02–4.97 (ddd,  $J$  = 2, 5, 11 Hz, 1H), 4.94–4.91 (dd,  $J$  = 4, 9.5 Hz, 1H), 4.72–4.66 (m, 1H), 3.76 (s, 3H), 3.54–3.47 (m, 1H), 3.47–3.40 (m, 1H), 3.16–3.11 (dd,  $J$  = 6, 14.4 Hz, 1H), 3.03–2.97 (dd,  $J$  = 7.6, 14.4 Hz, 1H), 2.44–2.28 (m, 2H), 1.76–1.66 (m, 1H), 1.48–1.40 (m, 1H), 1.04–1.02 (d,  $J$  = 7 Hz, 3H), 0.92–0.90 (d,  $J$  = 6.4 Hz, 3H), 0.88–0.87 (d,  $J$  = 6.4 Hz, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  172.8, 170.82, 170.76, 165.7, 158.5, 141.8, 138.6, 130.2 (2C), 128.5, 124.9, 116.5, 114.1 (2C), 76.8, 71.4, 55.2, 54.3, 42.4, 39.8, 36.1, 35.2, 34.2, 32.5, 24.5, 22.9, 21.5, 16.6; IR (film) 3420, 3300, 2990, 1740, 1675, 1525  $cm^{-1}$ ; MS (FAB+, TG/G) 515.3 (M + H); HRMS (FAB, TG/G) calcd for  $C_{28}H_{39}O_7N_2$  (M + H) 515.2757, found 515.2775.

**tert-Butyl (5S,6R,2E,7E)-5-[(2S)-2-[3-[(2R)-2-tert-Butoxycarbonylamino-3-(4-methoxyphenyl)propanoylamino]propanoyloxy]-4-methylpentanoyloxy]-6-methyl-8-phenyl-2E,7E-octadienoate (29).**<sup>36</sup> The *N*-Boc amino acid **6** (95 mg, 0.198 mmol) was dissolved in THF (3.8 mL), and then DIEA (43  $\mu$ L, 0.248 mmol), 2,4,6-trichlorobenzoyl chloride (34  $\mu$ L, 0.218 mmol), and DMAP were added. After 1.5 h, the alcohol **5** (30 mg, 0.099 mmol) dissolved in THF (200  $\mu$ L) was added dropwise. After 1 h, saturated aqueous  $NaHCO_3$  solution was added. The mixture was extracted with  $CH_2Cl_2$ . The combined organics were dried ( $MgSO_4$ ), filtered, and concentrated. Column chromatography (80:20 to 70:30 hexanes/EtOAc) provided the desired ester **29** as a colorless oil (54 mg, 71%):  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.32–7.18 (m, 5H), 7.13–7.10 (d,  $J$  = 8.5 Hz, 2H), 6.80–6.78 (d,  $J$  = 8.5 Hz, 2H), 6.76 (buried

m, 1H), 6.41–6.37 (d,  $J$  = 15.9 Hz, 1H), 6.02–5.95 (dd,  $J$  = 8.6, 15.9 Hz, 1H), 5.83–5.80 (d,  $J$  = 15.6 Hz, 1H), 5.45–5.43 (d,  $J$  = 8 Hz, 1H), 5.03–5.00 (m, 1H), 4.96–4.93 (dd,  $J$  = 4, 10 Hz, 1H), 4.36–4.31 (m, 1H), 3.75 (s, 3H), 3.54–3.52 (m, 1H), 3.48–3.41 (m, 1H), 3.15–3.10 (dd,  $J$  = 5.5, 14 Hz, 1H), 2.93–2.88 (m, 1H), 2.60–2.40 (m, 5H), 1.71–1.57 (m, 3H), 1.47 (s, 9H), 1.34 (s, 9H), 1.33 (buried, 1H), 1.10–1.08 (d,  $J$  = 6.9 Hz, 3H), 0.83–0.81 (d,  $J$  = 6.5 Hz, 3H), 0.78–0.76 (d,  $J$  = 6.5 Hz, 3H); other data as previously reported.<sup>36</sup>

**Desepoxyarenastatin A (4) from 29.**<sup>36</sup> Ester **29** (50 mg, 0.065 mmol) was dissolved in  $CH_2Cl_2$  (6 mL), and TFA (200  $\mu$ L) was added. After 1 h, toluene (2 mL) was added, and the reaction mixture was concentrated in vacuo. The resulting acid was dissolved in  $CH_3CN$  (6 mL), and DIEA (33  $\mu$ L, 0.19 mmol) and HBTU (28 mg, 0.075 mmol) were added. The reaction was stirred at room temperature for 45 min. Saturated aqueous  $NaHCO_3$  solution was added, and the aqueous phase was extracted with  $CH_2Cl_2$ . The combined organics were dried ( $MgSO_4$ ), filtered, and concentrated. Column chromatography (7:1 to 4:1  $CH_2Cl_2$ /acetone) provided **4** as an oil (24 mg, 65%):  $[\alpha]^{20}_D$  +27° (c 0.80,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.33–7.15 (m, 5H), 7.11–7.09 (d,  $J$  = 8.6 Hz, 2H), 7.05–7.02 (t,  $J$  = 5.5 Hz, 1H), 6.81–6.78 (d,  $J$  = 8.6 Hz, 2H), 6.73–6.66 (ddd,  $J$  = 4.7, 10.5, 15 Hz, 1H), 6.41–6.37 (d,  $J$  = 15.8 Hz, 1H), 6.03–5.96 (dd,  $J$  = 8.8, 15.8 Hz, 1H), 5.77–5.75 (d,  $J$  = 7.9 Hz, 1H), 5.75–5.71 (d,  $J$  = 15 Hz, 1H), 5.06–5.01 (ddd,  $J$  = 2, 6.6, 11 Hz, 1H), 4.91–4.88 (dd,  $J$  = 3.6, 10 Hz, 1H), 4.73–4.67 (m, 1H), 3.76 (s, 3H), 3.54–3.48 (m, 1H), 3.46–3.39 (m, 1H), 3.15–3.11 (dd,  $J$  = 6, 14.4, 1H), 3.04–2.98 (dd,  $J$  = 7.5, 14.4 Hz, 1H), 2.57–2.52 (m, 3H), 2.39–2.30 (m, 1H), 1.78–1.57 (m, 3H), 1.35–1.27 (m, 1H), 1.13–1.11 (d,  $J$  = 6.8 Hz, 3H), 0.73–0.72 (d,  $J$  = 6.4 Hz, 3H), 0.70–0.69 (d,  $J$  = 6.4 Hz, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  172.8, 170.9, 170.8, 165.6, 158.5, 141.7, 136.7, 131.8, 130.2 (2C), 128.6 (2C), 128.5, 127.5, 126.1 (2C), 125.0, 114.1 (2C), 76.6, 71.5, 55.2, 54.3, 42.2, 39.7, 36.4, 35.2, 34.2, 32.4, 24.3, 22.6, 21.2, 17.2; IR (film) 3365, 3260, 2940, 1725, 1710, 1665  $cm^{-1}$ ; MS (FAB, TG/G)  $m/z$  591.3 (M + H); HRMS (FAB, TG/G) calcd for  $C_{34}H_{43}N_2O_4$  (M + H) 591.3070, found 591.3069.

**Desepoxyarenastatin A (4) from 30.** Olefin **30** (17 mg, 0.033 mmol) was dissolved in  $CH_3CN$  (0.33 mL) in a dry sealed tube. The solution was flushed with argon, and then iodobenzene (4 mL, 0.036 mmol),  $Pd(OAc)_2$  (1.1 mg, 0.005 mmol), and TEA (46 mL, 0.330 mmol) were added. The tube was sealed and placed in a 80–85 °C oil bath with vigorous stirring overnight. After 20 h, the solution was filtered and purified by silica gel chromatography to obtain product **4** as a solid (3 mg, 31% based on recovered starting material) and unreacted starting material (6 mg).

**Arenastatin A (2).** Olefin **4** (5.0 mg, 8.5  $\mu$ mol) was reacted as previously reported<sup>36</sup> with dimethyldioxirane<sup>64</sup> to obtain a 2:1 mixture (*de* was determined by HPLC Phenomenex Hypersil 5  $\mu$ m, C18, 150  $\times$  3.2 mm, 254 nm, 3:2  $CH_3CN/H_2O$ , 0.5 mL/min, retention time:  $\beta$  = 7.2 min,  $\alpha$  = 7.9 min) of epoxide diastereomers (3.9 mg, 76%):  $[\alpha]^{20}_D$  +37° (c 0.10,  $CHCl_3$ ).<sup>7,36</sup>

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**Supporting Information Available:**  $^1H$  NMR spectra for compounds **2**, **4**–**7**, **9**, **13**, **14**, **16**, **18**–**25**, and **27**–**30**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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LETTERS

# ENANTIOSELECTIVE SYNTHESIS OF A 3'-DEPHENYLCRYPTOPHYCIN SYNTHON<sup>1</sup>

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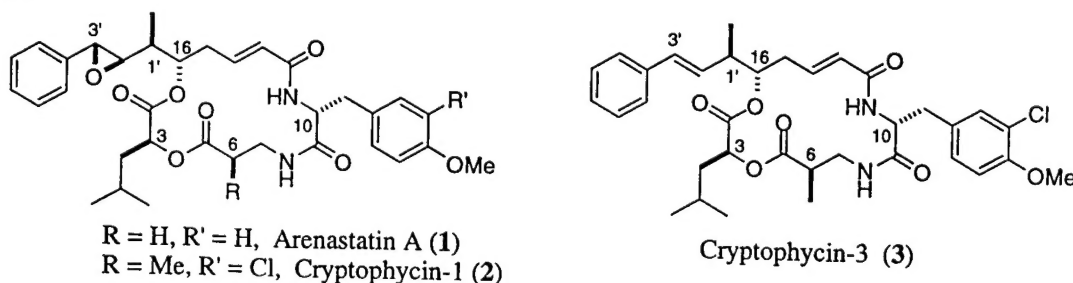
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**Abstract:** An enantioselective synthesis of *tert*-butyl (5*S*,6*R*)-(E)-5-*tert*-butyldimethylsilyloxy-6-methyl-2,7-octadienoate, a precursor for the synthesis of the antimitotic macrolides cryptophycin A and arenastatin A (cryptophycin-24), is presented. The key step in the reaction sequence features a crotyl boration that sets both stereocenters that become the C16 hydroxyl and C1' methyl in the cryptophycins. Homologation of the terminal olefin via a Heck reaction is presented. © 1998 Elsevier Science Ltd. All rights reserved.

The cryptophycins (Figure 1) are 16-membered macrolides isolated from the blue-green algae (cyanobacteria) *Nostoc* sp. GSV 224.<sup>2,3</sup> Arenastatin A (1, Figure 1), cryptophycin-24, had previously been isolated from the Okinawan marine sponge *Dysidea arenaria*.<sup>4</sup> Thus far, 25 compounds of this class have been identified.<sup>5,6</sup> Cryptophycin-1 (2), the most abundant component, was found to have significant tumor selective cytotoxicity against drug- and multiple drug-resistant tumor cells.<sup>3</sup> When administered intravenously, depsipeptide 2 was also very effective against subcutaneously transplanted solid tumors in mice.<sup>3</sup> The cytotoxic activity of cryptophycins is derived from their inhibition of tubulin polymerization into microtubules.<sup>7,8</sup> Additionally, they inhibit microtubule dynamics making them somewhat unique in their activity.<sup>9,10</sup>

Figure 1

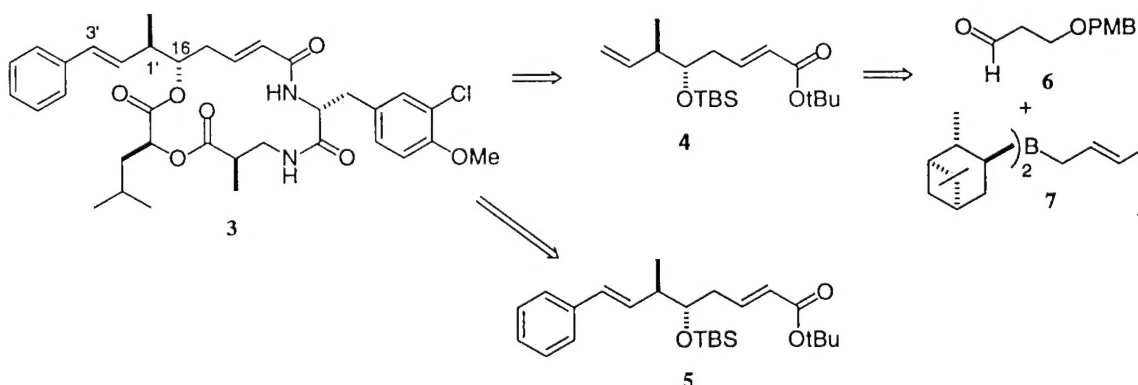


Initial knowledge of the structure-activity relationships (SAR) of the cryptophycins was obtained from natural products isolated from *Nostoc*.<sup>5,6</sup> Although natural variation occurred on the substituents of the macrocycle, alterations were absent from the C3' aromatic moiety. Through synthetic approaches, it has become

clear that the epoxide and the absolute and relative stereochemistry at the epoxide and at C16 and C1' are necessary for activity.<sup>10,11</sup> For example, compound **3**, a common intermediate in most of the total syntheses, is essentially inactive.<sup>5</sup> However, until recently nothing was known about the terminal aromatic group.<sup>11</sup> Several syntheses and formal syntheses from our group and others have been presented,<sup>12–17</sup> but none have addressed the practical modification of the C3' aromatic moiety.

Our approach (Scheme 1) involved development of a concise method in which both stereocenters were incorporated in a single step utilizing a readily scalable method such as the crotyl boration. Fragment **4**, as we envisioned, would allow for incorporation of a variety of aryl groups after macrocyclization for thorough SAR studies. In addition, it could be utilized for the direct synthesis of **5** for ongoing studies probing other regions of the cryptophycin structure. Herein we report our synthesis of synthon **4** originating from aldehyde **6** and (*E*)-crotyl borane **7** (Scheme 1) amenable to structure–activity studies at the C3' aromatic side chain. Additionally, we present the highly convergent and concise synthesis of building block **5**.

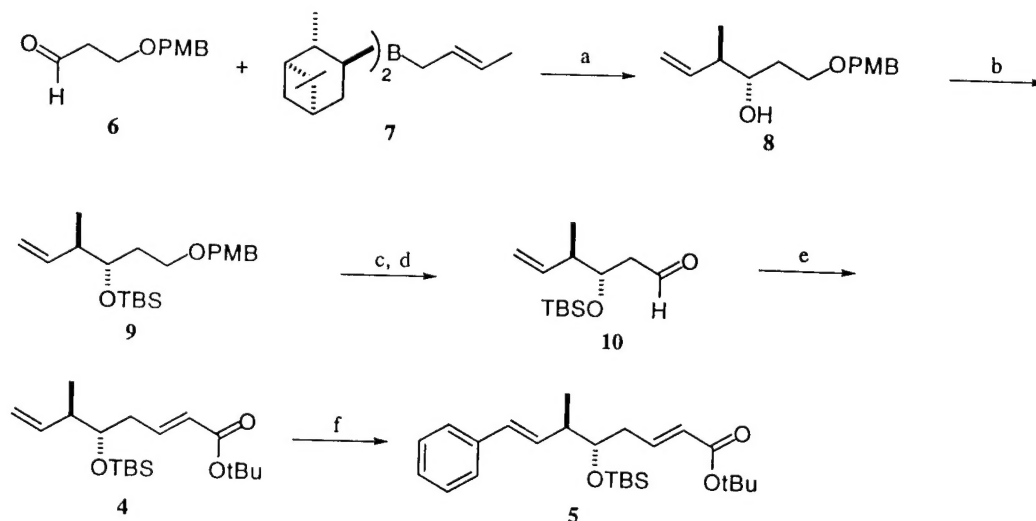
Scheme 1



The synthesis of octadienoate **4** was accomplished in seven steps from 1,3-propanediol (Scheme 2). The key step in our reaction sequence of segment **4** utilizes the crotyl boration<sup>18</sup> of aldehyde **6**<sup>19</sup> with **7** (prepared from (+)-*B*-methoxydiisopinocampheylborane) to generate the desired stereochemistry at the two chiral centers of **8** in 55% yield (91% ee).<sup>20</sup> Silyl protection of the secondary alcohol **8** with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and 2,6-lutidine proceeded at low temperature in 92% yield. Rapid deprotection of the *p*-methoxybenzyl ether with DDQ at 0 °C, followed by flash chromatography provided a mixture of *p*-methoxybenzaldehyde and the desired primary alcohol. The mixture was subjected to TPAP

oxidation<sup>21</sup> conditions in the presence of NMO producing aldehyde **10** after chromatography in 74% yield over two steps. The Horner–Emmons homologation to form the  $\alpha,\beta$ -unsaturated *tert*-butyl ester **4**<sup>22</sup> proceeded cleanly using *tert*-butyl diethylphosphonoacetate, DBU and LiCl. The octadienoate **4** was converted to the key synthon **5**<sup>23</sup> using a Heck reaction<sup>24</sup> with iodobenzene in 84% yield.

Scheme 2



a) THF, Et<sub>2</sub>O, -78 °C, 2 h, then HOCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, 55%, (91% ee); b) TBSOTf, 2,6-lutidine, THF, -78 °C, 10 min, 92%; c) DDQ, CH<sub>2</sub>Cl<sub>2</sub>, H<sub>2</sub>O, 0 °C, 15 min; d) TPAP, NMO, CH<sub>2</sub>Cl<sub>2</sub>, rt, 15 min, 74% over two steps; e) (EtO)<sub>2</sub>P(O)CH<sub>2</sub>C(O)OtBu, DBU, LiCl, CH<sub>3</sub>CN, rt, 2 h, 89%; f) PhI, Pd(OAc)<sub>2</sub>, Et<sub>3</sub>N, CH<sub>3</sub>CN, sealed tube, 83 °C, 18 h, 84%.

In conclusion, we have developed a convenient enantioselective route to the 3'-dephenyl synthon **4** of the cryptophycins. We also applied this approach to the rapid synthesis of fragment **5**. Application of octadienoate **4** to the total synthesis and structure–activity studies at the aromatic side chain will be reported in due course.

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20. The enantiomeric excess was determined by chiral HPLC comparing alcohol **8**, derived from (+)-*B*-methoxydiisopinocampheylborane (purchased from Aldrich chemical company) with alcohol ent-**8** derived from reaction with the (–)-borane: Chiralcel OD-H, 254 nm, 99:1 hexanes:isopropyl alcohol, 0.5 mL/min, retention time: ent-**8** = 32.2 min, **8** = 34.6 min.
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22. Compound **4**:  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.82–6.75 (dt,  $J$  = 8, 16 Hz, 1 H), 5.80–5.71 (buried, 1H), 5.74–5.70 (br d,  $J$  = 16 Hz, 1H), 5.03 (br s, 1H), 5.01–4.99 (br d,  $J$  = 9 Hz, 1H), 3.67–3.63 (m, 1H), 2.29–2.23 (m, 3H), 1.46 (s, 9H), 1.00–0.99 (d,  $J$  = 6.8 Hz, 3H), 0.88 (s, 9H), 0.033 (s, 3H), 0.027 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  165.7, 145.1, 140.1, 125.0, 115.1, 80.0, 74.9, 43.3, 36.8, 28.1 (3C), 25.8 (3C), 18.1, 15.3, –4.4, –4.6; HRMS (FAB) calcd for  $\text{C}_{19}\text{H}_{37}\text{O}_3\text{Si}$  ( $M + \text{H}$ ) 341.2512, found 341.2514.
23. Compound **5** was also synthesized by an alternate route [see ref 17,  $[\alpha]_D^{+69^\circ}$  ( $c$  0.73,  $\text{CHCl}_3$ ):  $[\alpha]_D^{+64^\circ}$  ( $c$  0.73,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.37–7.17 (m, 5 H), 6.86–6.76 (dt,  $J$  = 8, 16 Hz, 1 H), 6.38–6.33 (d,  $J$  = 16 Hz, 1H), 6.18–6.10 (dd,  $J$  = 8, 16 Hz, 1H), 5.74–5.69 (d,  $J$  = 16 Hz, 1H), 3.74–3.69 (app q,  $J$  = 6 Hz, 1H), 2.47–2.40 (m, 1H), 2.32–2.27 (m, 2H), 1.45 (s, 9H), 1.09–1.06 (d,  $J$  = 7 Hz, 3H), 0.88 (s, 9H), 0.033 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  165.8, 144.8, 137.6, 132.0, 130.4, 128.5 (2C), 127.0, 126.0 (2C), 125.1, 80.0, 75.1, 42.8, 37.3, 28.1 (3C), 25.9 (3C), 18.1, 16.1, –4.4, –4.5; HRMS (FAB) calcd for  $\text{C}_{25}\text{H}_{41}\text{O}_3\text{Si}$  ( $M + \text{H}$ ) 417.2825, found 417.2848.
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